Hepatic Encephalopathy: Role of Benzodiazepine Receptor Ligands

Medical Grand Rounds

University of Texas
Southwestern Medical Center

David K. Spady M.D.

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Introduction

Hepatic encephalopathy is a complex neuropsychiatric syndrome associated with hepatocellular failure and/or increased portal-systemic shunting of blood. Hepatic encephalopathy is characterized by symptoms ranging from very subtle subclinical abnormalities of intellectual and motor function to coma(1-4). The syndrome usually occurs in one of three clinical settings (Table I). One is fulminant hepatic failure, which is the term used when hepatic encephalopathy occurs within a few weeks of the first evidence of liver disease. Common causes of fulminant hepatic failure include viral and alcoholic hepatitis, drug reactions and acetominophen overdose. A second clinical variant is acute hepatic encephalopathy complicating chronic liver disease and in this setting, precipitating factors play an important role. Common precipitating factors include GI bleeding, infections, fluid/electrolyte imbalance, sedatives and excess alcohol. Prognosis for recovery is excellent in these patients and depends primarily on the prompt identification and treatment of the precipitating event. A third clinical pattern is characterized by a progressive, prolonged persistence of neuropsychiatric symptoms. The term chronic portal-systemic encephalopathy is often applied to this variant of the syndrome. Precipitating factors do not play a major role in this clinical setting although these patients are generally intolerant of large protein loads. Whether or not the pathogenesis of encephalopathy in these three clinical settings is the same is not known.

Clinically unrecognized impairment of mental function is frequent in patients with chronic liver disease. Indeed, the majority of patients with chronic liver disease who display no overt behavioral, neurological or EEG changes will have abnormal scores in psychomotor tests. In Germany, 85% of patients with chronic liver disease and portal hypertension were judged unfit or questionably fit to drive a car based on extensive pyschomotor testing(5).

Table I. Spectrum of Hepatic Encephalopathy

Clinical Setting	% Survival
Acute	
• Fulminant hepatic failure	20
• Cirrhosis with precipitant	70-80
Chronic	
• Portal-systemic encephalopathy	100

Hepatic encephalopathy is unusual if liver function is normal; however, increased portal-systemic shunting of blood may lead to encephalopathy in the absence of obvious liver disease. The reversible nature of the brain dysfunction, at least in the early stages, and the diffuse involvement are compatible with the concept of a metabolic encephalopathy. Pathologic changes may be observed in patients dying of hepatic encephalopathy but are not considered an essential component of the syndrome. Up to 50% of patients with fulminant hepatic failure and prolonged coma develop cerebral edema and increased intracranial pressure. In contrast, cerebral atrophy is common in cirrhotic patients with chronic recurrent hepatic encephalopathy. No significant changes in neuron structure in hepatic encephalopthy have been observed at the light or electron microscopic level although an increased number of astroglial cells are frequently seen.

Clinical Manifestations

The picture of hepatic encephalopathy is complex and includes a broad spectrum of fluctuating psychiatric and neurological abnormalities. Clinically, hepatic encephalopathy is usually divided into four stages based on mental state and neuromuscular function as outlined in Table II. Personality and psychiatric changes are common early on but may be more obvious to family members and friends than to the physician. With progression, there is eventual loss of consciousness. Motor abnormalities range from mild incoordination, asterixis and hyperreflexia to decerebrate posturing and loss of oculovestibular responses. Delirium and seizures are atypical but may occur in hepatic encephalopathy due to fulminent hepatic failure.

Table II. Clinical Stages of Hepatic Encephalopathy

Stage	Mental state	Neuromuscular state
I	Confused Altered mood or behavior	Mild incoordination
II	Drowsy Inappropriate behavior	Asterixis Ataxia Dysarthria
III	Somnolent but rousable Disoriented	Asterixis Abnormal reflexes
IV	Coma	No response to painful stimuli Decerebrate

EEG changes occur very early even before psychological or biochemical disturbances. EEG changes, while not pathognomonic of hepatic encephalopathy, are fairly characteristic(6). At first, the normal alpha rhythm (9 to 12 cycles per second) is disturbed by random waves occurring at a frequency of 5 to 7 cps (theta waves). These are followed by the appearance of larger triphasic waves with a frequency of 4 to 5 cps, which displace some of the theta waves. As coma deepens, large random and arrhythmic waves with a frequency of 2 to 3 cps (delta waves) appear and dominate the EEG. As encephalopathy improves, the sequence of slowing is reversed.

Laboratory tests are of value in the differential diagnosis of encephalopathy and in identifying precipitating factors. However, no single laboratory test is particularly useful in diagnosing or assessing hepatic encephalopathy. Isolated serum ammonia levels do not correlate well with the stage of encephalopathy although serial determinations may be useful for following the course of the condition. Of all the laboratory tests correlated with hepatic encephalopathy, CSF glutamine and a-ketoglutaramate appear to correlate best with the presence and grade of coma(4).

Pathogenesis

The pathogenesis of hepatic encephalopathy is thought to involve the accumulation of neuroactive and potentially comagenic substances in the systemic circulation that are normally cleared and metabolized by the liver. The alternative hypothesis--that in liver failure there is diminished hepatic synthesis of a substance necessary for normal brain function--appears unlikely based on cross-circulation studies carried out in animals(7). Hepatic encephalopathy is associated with changes in the concentrations of a large number of compounds in plasma, CSF and brain(4). These findings have led to the formulation of several hypotheses of the pathogenesis of hepatic encephalopathy, none of which has been conclusively validated. Given the range of metabolic and neuropsychiatric abnormalities seen in liver failure, it is likely that multiple factors contribute to the etiology of hepatic encephalopathy.

In general, investigations into the pathogenesis of hepatic encephalopathy have focused on three potential mechanisms. The first involves putative neurotoxins of which ammonia has received the most attention. A second line of investigation has focused on the complex alterations in amino acid metabolism in liver disease and the role of amino acids as neurotransmitters in the brain. More recently, it has been proposed that alterations in gamma amino butyric acid (GABA)-mediated

neurotransmission may be responsible for the central nervous system dysfunction in liver disease(8, 9).

Role of Ammonia Toxicity

Of all the compounds potentially responsible for hepatic encephalopathy, ammonia has been the most widely investigated. Ammonia is produced in several organs but the major source is the gut(10-12). About half of intestinal ammonia production is derived from the colon and half from the small intestine. Ammonia formation in the small intestine is accounted for by the metabolism of glutamine, the major respiratory fuel of this organ. Colonic ammonia production is due predominantly to the hydrolysis of proteins, amino acids and urea under the influence of colonic bacteria. During starvation or catabolic states, large amounts of ammonia are also derived from the breakdown of body proteins. Normally, more than 95% of ammonia is cleared by the liver where it is converted to urea in the urea cycle. About 2% is excreted in the urine and the remainder is detoxified in a variety of tissues by the amination of glutamate to glutamine. Portal-systemic shunting or hepatocellular failure permits ammonia to enter the systemic circulation. Unionized ammonia (about 5% of total blood ammonia at physiologic pH) is taken up by a variety of tissues, including the brain, in direct proportion to plasma levels. In patients with advanced liver disease or portal-systemic shunting, skeletal muscle is the most important site of ammonia detoxification(10).

Clinical evidence suggesting a role for ammonia in hepatic encephalopathy is based on the following observations. As discussed above, ammonia accumulates in liver disease and readily enters the brain. Several early reports described the development of encephalopathy in some patients with severe liver disease or portal-caval shunt after chronic ingestion of nitrogenous compounds(13-15). Encephalopathy is a prominent feature of inherited hyperammonemic syndromes although plasma ammonia levels are usually far higher than those associated with liver disease(16). Finally, therapies that decrease intestinal ammonia absorption are often followed by improvement of hepatic encephalopathy.

Nevertheless, there are a number of observations that are inconsistent with a predominant role for ammonia in hepatic encephalopathy. Thus, the acute administration of ammonia to patients with severe liver disease does not consistently produce encephalopathy and, in general, does not produce EEG changes characteristic of hepatic encephalopathy even though high plasma ammonia levels are achieved(17, 18). In normal animals, progressive acute ammonia toxicity is

characterized by a brief lethargic state followed by seizures and postictal coma(19, 20). Although seizures are common in congenital hyperammonemia syndromes, they are unusual in hepatic encephalopathy. Moreover, the electrophysiological effects of ammonia in normal animals or on preparations of normal neurons are quite different from the changes that occur in animal models of hepatic encephalopathy. At the relevant concentrations, ammonia has been shown to cause disinhibition in neural circuits by blocking the outward extrusion of chloride ions(21). This is consistent with the tendency to seizures in acute ammonia toxicity. In contrast, neural circuits are inhibited in models of hepatic encephalopathy(9).

It has been proposed that ammonia interferes with cerebral energy metabolism by removing α-keto glutarate from the Krebs cycle and diverting it into the glutamine pathway. Indeed, hepatic encephalopathy is associated with reductions in cerebral blood flow and in the consumption of glucose and oxygen. However, it is now apparent that these changes in cerebral energy metabolism reflect a decreased demand for energy rather than the primary cause of hepatic encephalopathy(4, 22, 23).

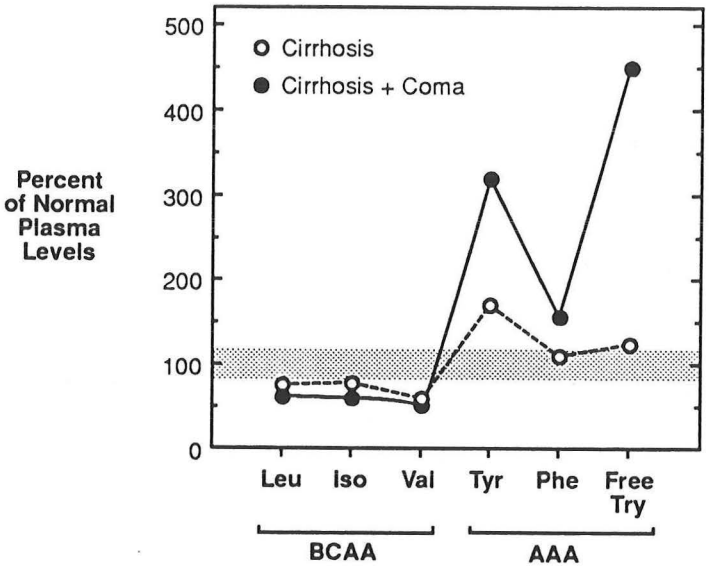
In addition to ammonia, there are a large number of other compounds that accumulate in the plasma of patients with liver failure (fatty acids, mercaptens, phenols) that are neurotoxic if administered in large enough quantities. It has been proposed that these compounds may act synergistically to produce hepatic encephalopathy--a hypothesis that is difficult to prove or disprove(4).

Thus, there is a strong clinical impression that hepatic encephalopathy is due, at least in part, to nitrogenous substances of gut origin that reach the brain either through portal systemic shunts or by failure of removal by the diseased liver. However, despite decades of research, the identity of the toxic substance(s) and the mechanisms involved in brain dysfunction remain unresolved.

Role of Amino acid Imbalance and false neurotransmitters

Plasma amino acid levels are consistently abnormal in patients with liver failure (Fig. 1). In cirrhosis, the concentrations of the free aromatic amino acids, tyrosine, phenylalanine and tryptophan, are increased whereas the branched-chain amino acids, valine, leucine and isoleucine, are decreased(24, 25). The molar ratio between the branched-chain and the aromatic amino acids is often reduced from the normal value of >3 to levels <1. It has been postulated that increased plasma concentrations of the aromatic amino acids may cause encephalopathy due either to direct neurotoxicity or to the fact that these amino acids are the direct precursors

Fig. 1. Alterations in plasma amino acid profile in cirrhosis (Ref. 25)



of the monoamine neurotransmitters (serotonin, epinephrine, norepinephrine and dopamine) as well as several weak or "false" neurotransmitters (octopamine and phenylethanolamine) that may replace or compete with catecholamines(4, 24).

Some aromatic amino acids, particularly tryptophan, have been found to be directly neurotoxic, although at extremely high doses. Ingestion of large amounts of tryptophan by patients may result in headache, dizziness, drowsiness, disturbances of gait and abnormal reflexes; however these changes occur at plasma concentrations much greater than those seen in hepatic encephalopathy(26). Induction of coma in normal dogs by the simultaneous intracarotid infusion of massive amounts of tryptophan and phenylalanine has been reported(27) but again, the CSF concentrations achieved were far higher than those seen in humans with hepatic encephalopathy(25).

Tryptophan is the direct precursor of serotonin. Elevated levels of serotonin have been postulated to be involved in the CNS depression observed in hepatic encephalopathy and could result from the increased brain levels of tryptophan observed in liver failure(25). Whether elevated brain levels of serotonin or its metabolites actually occur in hepatic encephalopathy (compared to liver disease without encephalopathy) is uncertain; however, recent studies in several animal models of hepatic encephalopathy indicate no correlation between alterations in brain serotonin metabolism and neurological status(26).

Elevated plasma levels of the aromatic amino acids phenylalanine and tyrosine are associated with increased levels of the "false" neurotransmitters, octopamine and phenylethanolamine. Octopamine and phenylethanolamine, both derived from phenylalanine and tyrosine, may deplete catecholamine neurotransmitter stores by displacing norepinephrine and dopamine from synaptic vesicles. Since octopamine and phenylethanolamine act as weak neurotransmitters, an accumulation of these amines in the central nerve endings could result in diminished neurotransmission. However, by direct infusion of octopamine into the lateral cerebral ventricle of rats, brain octopamine levels have been raised > 20,000-fold and brain dopamine and norepinephrine reduced to < 10% of controls without causing coma(28). Moreover, in autopsy studies, octopamine levels are not increased nor catecholamine levels decreased in brain tissues of cirrhotic patients with hepatic encephalopathy(29, 30).

Overall, the data suggest that the altered amino acid metabolism associated with liver failure does not play a major role in hepatic encephalopathy. Clinically, despite the marked decrease in the plasma branched-chain to aromatic amino acid ratio observed in chronic liver failure, this ratio is not a reliable indicator of the onset or severity of encephalopathy and correlates much better with the severity of liver disease than with the presence or severity of encephalopathy. Moreover, as discussed below, therapeutic strategies aimed at correcting the abnormal plasma amino acid profile have not been successful in ameliorating hepatic encephalopathy.

Role of the GABA-Benzodiazepine Receptor Complex

The network of neurons that make up the central nervous system operates by balancing inhibitory and excitatory chemical messages. Over 100 neurotransmitters are known to participate in this complex process and depending on whether thy depolarize or hyperpolarize receptive neurons, the transmitters are classified as excitatory or inhibitory. GABA is the principal inhibitory neurotransmitter of mammalian brain and more than one-third of central synapses are GABAergic(31, 32). Since each neuron may have thousands of synaptic connections, it is likely that all neurons in the central nervous system are inhibited by GABA.

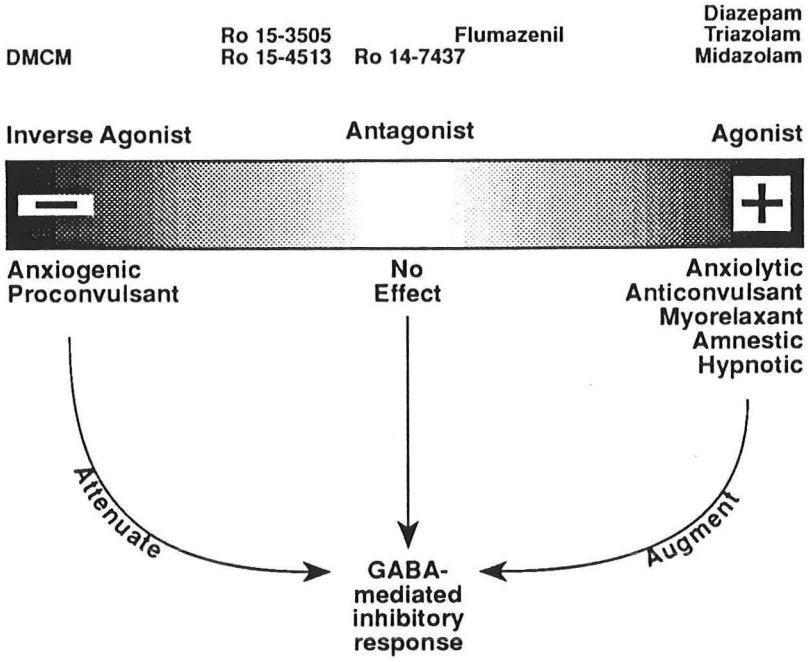
In 1982 Schafer and Jones hypothesized that hepatic encephalopathy could be mediated by activation of GABAergic mechanisms(33). This hypothesis was based on a series of experiments carried out in rabbits with acute hepatic failure. The initial objective of these studies was to characterize the electrophysiologic changes in the brain associated with hepatic encephalopathy and other drug- and toxin-induced

encephalopathies. It was noted that coma of different etiologies was associated with distinctive and reproducible changes in the pattern of visual evoked responses. (Visual evoked responses occur in response to repeated flashes of light and are derived from the EEG recorded over the occipital region of the brain.) The induction of hepatic encephalopathy in these animals was associated with a pattern of visual evoked responses that was almost identical to those associated with coma induced by benzodiazepines or barbiturates--drugs whose sedative-hypnotic effects are mediated by the GABAergic system(34). Furthermore, the abnormal pattern of visual evoked responses could be reversed by the administration of a GABA receptor antagonist and these improvements in electrophysiologic abnormalities were associated with ameliorations in the neurological manifestations of hepatic encephalopathy(35). These findings first suggested an involvement of GABAergic transmission in hepatic encephalopathy and prompted a search for abnormalities of the GABA receptor or altered metabolism of its ligands that might contribute to hepatic encephalopathy.

GABA mediates neuronal inhibition by binding to the $GABA_A$ receptor which operates a chloride channel embedded in the plasma membranes of target neurons(36). The binding of GABA triggers the opening of the chloride channel thereby permitting the passage of chloride ions into the neuron, hyperpolarizing the membrane and rendering it less likely to depolarize. These events are the basis of GABA-mediated inhibition of neurotransmission. Pharmacologically, the the $GABA_A$ receptor complex includes the chloride channel, the GABA binding site and a variety of binding sites for pharmaceutically significant drugs, such as the benzodiazepines and barbiturates, which interact allosterically with the GABA agonist site or the receptor channel(37). Structurally, the $GABA_A$ receptor complex is thought to consist of at least 3 distinct protein subunits termed α, β and γ (which themselves exist in multiple subtypes, ie., α1-6, β1-3, γ1-2)(38). The minimum subunit structure appears to contain α and β subunits in a form which is essentially insensitive to benzodiazepines(39, 40). The more usual benzodiazepine-sensative forms also contain the γ subunit(41, 42). Based on the molecular weights of the subunits and the entire complex, it is thought that a minimum of five subunits (for example, 2 α, 2 β and 1 γ) are necessary to form a fully functioning complex. The potential for numerous combinations of these subunits is thought to account for the multiple forms of the receptor that are seen in different locations in the brain or at different stages of development.

In the original GABA hypothesis, hepatic encephalopathy was postulated to result from the actions of GABA or GABA-like compounds derived from the gut and gaining access to the brain in liver failure(33).

Fig. 2. Spectrum of activities of benzodiazepine receptor ligands (Ref. 9)



However, most investigators were unable to demonstrate an increase in plasma or brain GABA levels or in the number or affinity of brain GABA receptors(43-45) and attention shifted to the role of the benzodiazepine receptor when it was found that the benzodiazepine receptor antagonist flumazenil (Ro 15-1788) partially reversed the electrophysiologic abnormalities and transiently improved the neurologic status in animals with hepatic encephalopathy at doses that had no effect in normal animals(35).

The basic function of the benzodiazepine receptor is to modulate GABA receptor gating of the chloride channel(37). Thousands of compounds have been synthesized or identified that interact with the benzodiazepine receptor. These ligands do not control chloride conductance directly but merely modulate the potency (without altering the maximal effect) of GABA. Because of the modulatory nature of this site, drugs that interact there generally have a high therapeutic index. Ligands that bind to the benzodiazepine receptor are classified according to their effect on GABA-mediated chloride conductance (Fig. 2). The first class of ligands is the benzodiazepine agonists (such as diazepam and midazolam). Occupation of the benzodiazepine receptor by an agonist augments the hyperpolarizing action of GABA leading to neural inhibition. These ligands have remarkable specificity for the benzodiazepine receptor

and interaction with this receptor is thought to account entirely for the well known anxiolytic, anticonvulsant and sedative-hypnotic effects of the benzodiazepine agonists(46).

The second type of benzodiazepine receptor ligand is the inverse agonist. Drugs of this type reduce GABAergic neurotransmission. These agents produce pharmacologic effects that are opposite to those of benzodiazepine agonists and include anxiety, discontent, increased muscle tension and seizures. One drug that is a partial inverse agonist has the property of partially reversing the sedative-hypnotic effects of ethanol(47, 48).

The third class of benzodiazepine receptor ligand is the antagonists. These drugs have minimal intrinsic activity (agonist or inverse agonist) at the benzodiazepine receptor and competitively antagonize benzodiazepine agonists and inverse agonists(49). Flumazenil (Ro-1788) is the prototype of this class of drugs and its actions would be analogous to the effect of naloxone at opiate receptors. Flumazenil competitively and reversibly inhibits the binding of benzodiazepine receptor ligands and appears not to interact with any other receptor. Nor does it block the actions of GABA, GABAmimetics, or barbiturates on the $GABA_A$ receptor complex. Flumazenil blocks essentially all of the specific behavioral and physiological effects of benzodiazepine receptor agonists, including their anxiolytic, sedative, hypnotic, amnestic and muscle relaxant properties. Flumazenil has little intrinsic activity; however, at high doses or concentrations it can produce a weak, benzodiazepine agonist-like effect consistent with its classification as a weak partial agonist.

The natural or physiologic ligand(s) for the benzodiazepine receptor is not known. Since the discovery of the benzodiazepine receptor(50), a number of substances have been identified in the brain that have measurable affinities for the benzodiazepine receptor; however, none of these has conclusively been shown to be a physiologically relevant ligand for the benzodiazepine receptor. Low levels of diazepam and metabolites have been detected in the brains of animals and from humans who died before the introduction of benzodiazepines but these are thought to arise from dietary sources(51). Potent and specific benzodiazepine receptor antagonists produce few neurobehavioural effects in normal animals or people suggesting that little, if any, endogenous benzodiazepine ligand is present under normal physiological conditions, or that its function is too subtle for reversal of its effect to be detected.

Benzodiazepine receptor antagonists have been shown to reverse the electrophysiologic abnormalities and to improve the behavioral manifestations of hepatic encephalopathy in animal models of fulminant hepatic failure(35, 52). In addition, single neurons in brain slices from animals with hepatic encephalopathy are 3-5 times more sensitive to

Fig. 3. Plasma benzodiazepine levels in patients with hepatic encephalopathy (Ref. 55)

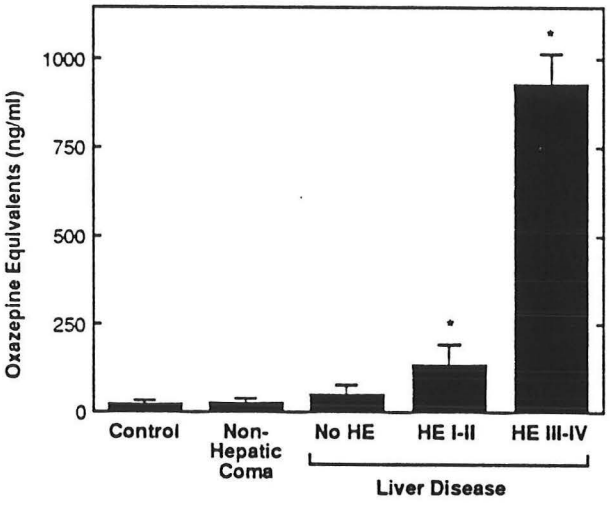
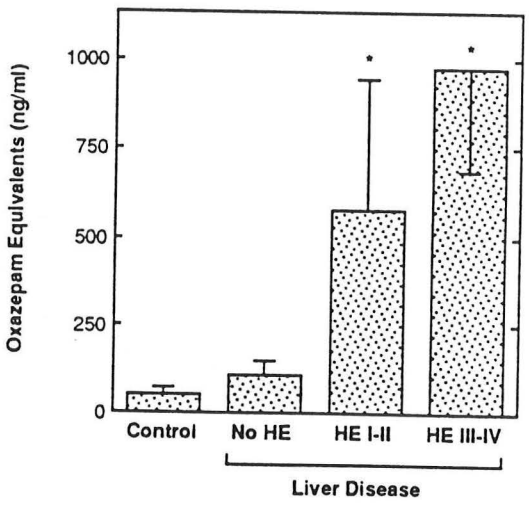


Fig. 4. Urinary benzodiazepine levels in patients with hepatic encephalopathy (Ref. 55)



depression by benzodiazepine receptor agonists than neurons from control animals and, in contrast to control neurons, are excited by the application of benzodiazepine receptor antagonists(53). Finally, as discussed below, benzodiazepine receptor antagonists appear to reverse the electrophysiologic abnormalities and improve the neurologic status in the majority of patients with hepatic encephalopathy.

Potential explanations for improvement of hepatic encephalopathy by benzodiazepine receptor antagonists include an increase in the number or affinity of benzodiazepine receptors or the presence of elevated brain levels of a benzodiazepine receptor agonist. In light of the almost universal finding that the number and affinity of benzodiazepine receptors is unchanged in human and experimental hepatic encephalopathy(43, 45, 54), attention has been turned to the later possibility, ie., that the improvement of hepatic encephalopathy by benzodiazepine antagonists is due to increased levels of an endogenous ligand.

Recently, plasma (Fig. 3) and urine (Fig. 4) benzodiazepine activity was found to be increased 10-30 fold in patients with hepatic encephalopathy and to correlate with the stage of encephalopathy(55). None of the patients had taken benzodiazepines for at least three months. These plasma and urine concentrations are well within the range that is detected by routine drug screens. A ~5-10 fold elevation of benzodiazepine receptor ligands has also been reported in the CSF (Fig. 5) of patients with hepatic encephalopathy compared to controls or to patients with liver disease but no encephalopathy(55, 56).

Fig. 5. CSF benzodiazepine levels in patients with hepatic encephalopathy (Ref. 56)

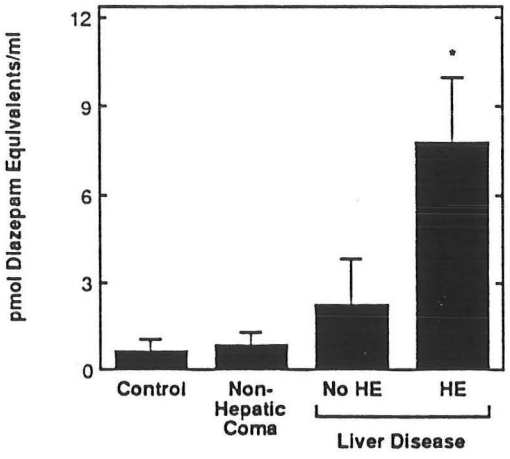
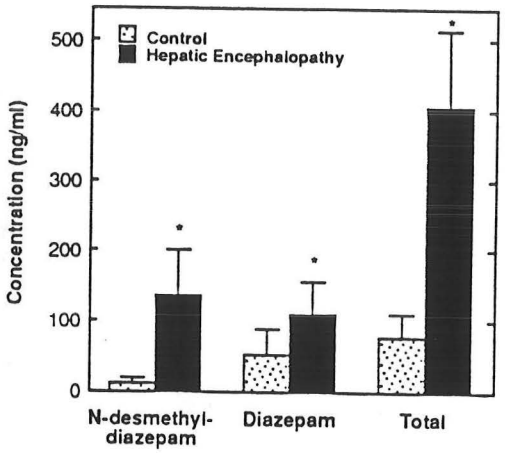


Fig. 6. Brain benzodiazepine levels in humans dying of FHF (Ref. 57)



In a recent autopsy study, the concentration of benzodiazepine receptor ligands in the frontal lobes was determined in patients dying of fulminent hepatic failure and compared to a group of patients dying of cardiovascular disease or trauma(57). Careful review of histories and charts showed no recent benzodiazepine use in any patient. The concentration of total benzodiazepine receptor ligands in the brain samples from patients with hepatic encephalopathy was more than five times higher than in samples from the control patients (Fig. 6) and the presence of diazepam and n-desmethyldiazepam (an active metabolite of several benzodiazepines) was confirmed by GC-mass spectrometry. The patients with fulminant hepatic failure were not a homogeneous population in that ~60% had elevated brain levels of benzodiazepine activity whereas the remainder had levels no different than controls. This may explain why only ~60% of patients respond to benzodiazepine receptor antagonists as discussed below.

The concentrations of benzodiazepine receptor ligands in brain, CSF, plasma and urine are generally in the range that would be expected after the administration of low doses of diazepam and thus appear lower than those required to cause overt encephalopathy. Nevertheless, in view of the known hypersensitivity of patients with severe liver disease to benzodiazepines, these low levels may be sufficient to exert an important effect.

The origin of the benzodiazepines in patients with hepatic encephalopathy is unknown. It is possible that some of these patients had received benzodiazepines despite the efforts of the physicians to identify their use. This seems unlikely, however, since animal studies were

performed before proceeding to human studies and similar changes in brain, CSF, plasma and urine benzodiazepine ligands were seen in animal models of hepatic encephalopathy(54, 58, 59). Furthermore, the pattern of metabolites is not typical of that seen with exogenous administration of benzodiazepines. Although a transformed neuroblastoma cell line has been reported to synthesize benzodiazepines(60), there is little evidence that halogenated benzodiazepines are synthesized by mammalian tissues or by gut bacteria. Thus, for the moment, the diet remains the only obvious source of the benzodiazepines seen in these studies. Diazepam and metabolites of the benzodiazepines are known to occur in a wide variety of foods including wheat and potatoes(61), milk(62), and soy beans, rice and mushrooms(51). Benzodiazepine levels increase 5-8 fold during germination of wheat and potatoes suggesting that these plants biosynthesize benzodiazepines for use as growth regulators(61). However the quantities of benzodiazepines in food are small and the biological role and clinical relevance have not been elucidated.

In summary, the majority of patients with hepatic encephalopathy who otherwise have no history of exposure to benzodiazepines appear to have elevated levels of benzodiazepines in the urine, serum, CSF and brain. Normally, benzodiazepines are cleared and metabolized by the liver. Severe liver disease presumably allows levels in the systemic circulation to rise. Levels in blood and urine are frequently high enough to detect with routine drug screens and are in the range seen after an anxiolytic dose of a benzodiazepine. These levels may be sufficient to contribute to encephalopathy as suggested by a response to benzodiazepine receptor antagonists in many patients with hepatic encephalopathy.

Therapy

General Measures

Current management of hepatic encephalopathy is directed at identifying and correcting any precipitating factors and minimizing the absorption of nitrogenous substances from the gut. Potential precipitating factors can be identified in the majority of cirrhotic patients with acute hepatic encephalopathy. These include electrolyte and acid-base disturbances (frequently related to diuretics, diarrhea or vomiting), infections (pulmonary, urinary, peritoneal), GI bleeding, azotemia, constipation, and the use of sedative-hypnotic drugs. These should be specifically sought and treated.

The diagnosis of hepatic encephalopathy is usually readily apparent based on the clinical pattern of neuropsychiatric changes occurring in a

patient with known liver disease. However, if liver disease is unsuspected or if the presentation is atypical the differential diagnosis broadens to include space-occupying intracranial lesions, intracranial infections, toxic or other metabolic encephalopathies and various psychiatric disorders. In alcoholics, alcohol withdrawal, Wernicke's encephalopathy and subdural hematomas should be considered.

In patients admitted to the hospital with acute hepatic encephalopathy, ingestion of protein and other nitrogenous substances is reduced or stopped altogether. The bowel is evacuated by administering enemas or cathartics, and glucose (at least 1500 calories per day), which reduces protein catabolism, is provided as a source of energy. As discussed below, lactulose is administered orally or by enema with careful monitoring of fluid and electrolyte status to avoid hypernatremia. A short course of a broad spectrum antibiotic, usually neomycin 1 g four times a day, is generally recommended. As encephalopathy resolves, protein is added back to the diet as tolerated. Positive nitrogen balance is achieved with 40-60 g protein per day--an amount that is well-tolerated by most patients(63). Whether an attempt should be made to alter the quality of dietary protein is debatable(64-67). The apparent hierarchy of encephalopathogenic potential for dietary protein is meat > dairy > vegetable protein(64). Most likely the quality of dairy and vegetable protein matters less than the cathartic action of the accompanying dietary fiber or lactose (in lactase-deficient patients).

Lactulose

Lactulose is a synthetic disaccharide composed of galactose and fructose. Lactulose has been shown to be as effective as neomycin in the treatment of hepatic encephalopathy and is currently standard treatment for hepatic encephalopathy(68, 69). Like neomycin, lactulose is thought to exert its beneficial effects by reducing the absorption of nitrogenous substances from the gut. Lactulose is not absorbed or metabolized in the upper intestinal tract but is degraded by enteric flora in the lower gut to several organic acids, primarily lactate and acetate. These acids lower the luminal pH from around pH 7 to pH 5 or less and, in addition, produce an osmotic diarrhea. Metabolic alterations that accompany the administration of lactulose include a 2 to 4-fold increase in stool weight, a 2 to 4-fold increase in fecal nitrogen excretion (largely in the bacterial fraction) and a variable decrease in plasma ammonia.

The increase in fecal nitrogen excretion during lactulose therapy is thought to be due to one or more of the following mechanisms. First, the drop in fecal pH creates a pH gradient which favors the trapping of

ammonia ion and other amphoteric, nitrogenous toxic substances in the acidified colonic contents(70). Secondly, by reducing intestinal transit time, the osmotic diarrhea may decrease the time available both for the production and/or absorption of toxins from the gut. Finally, the presence of metabolizable carbohydrates in the colon promotes increasing ammonia nitrogen incorporation into bacterial protein, with a resultant decrease in urea and ammonia production(71, 72). In this regard, colonic bacteria prefer lactulose to blood when both are present so that lactulose may be of particular value in hepatic encephalopathy induced by GI bleeding(73).

The dose of lactulose is 10-30 ml three to four times a day and is adjusted to produce two to three semi-solid stools per day. Lactulose is well-tolerated(68, 69) but must be administered with caution. Overzealous use can produce severe osmotic diarrhea with loss of free water, hemoconcentration and hypernatremia(74). Up to 20% of patients complain of nausea, flatulence and abdominal cramping which usually resolves without discontinuing the drug. Patients also complain about the intense sweetness of the syrup and the unpredictable timing of catharsis. Lactitol is a second generation disaccharide (composed of galactose and sorbitol) that is produced in chemically pure, crystalline form and is dispensed as a powder. This compound is available in Europe and Canada where it has been shown to be equally, if not more, effective and better tolerated than lactulose(75).

Antibiotics

The rationale for the use of antibiotics in hepatic encephalopathy is to reduce the absorption of nitrogenous substances from the gut. Neomycin has long been used in the treatment of hepatic encephalopathy because of its poor absorption from the gut; however, the same effect may be achieved by the administration of any of several broad spectrum antibiotics including tetracycline, metronidazole and vancomycin(76, 77). Antibiotics are thought to work by decreasing the concentration of urease-containing bacteria in the colon, thus decreasing the production of ammonia. The effects of antibiotics and lactulose may be additive in acute hepatic encephalopathy(72, 78).

Because antibiotics are no more effective than lactulose in the treatment of hepatic encephalopathy and carry an increased risk of toxicity, they are no longer the primary therapy for chronic hepatic encephalopathy. Long-term neomycin treatment can cause significant oto-, neuro- or nephrotoxicity(79), malabsorption(80) and pseudomembranous colitis(81). Nevertheless, in refractory cases, neomycin is sometimes administered along with lactulose.

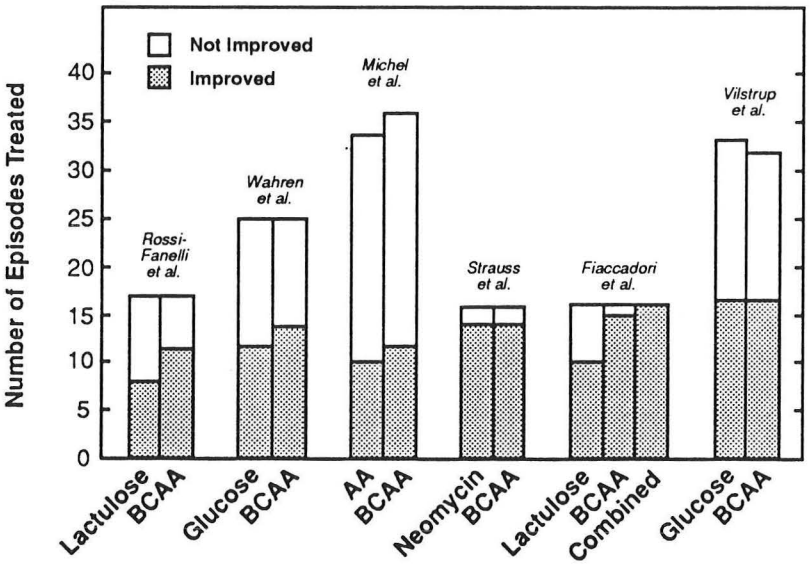
Branched-chain amino acids

As discussed above, the plasma amino acid profile is consistently abnormal in patients with cirrhosis with the most obvious change being a decrease in the branched-chain amino acids (Leucine, isoleucine and valine) relative to the aromatic amino acids (tyrosine, phenylalanine and tryptophan). Based on the hypothesis that such changes could lead to altered neurotransmission and encephalopathy, and on the observation that the abnormal plasma amino acid profile could be temporarily improved by administering branched-chain amino acids, a large number of studies were undertaken to investigate the value of branched-chain amino acids in the treatment of acute and chronic hepatic encephalopathy. The subject is confusing and controversial, even if only the randomized controlled trials are considered. The difficulties arise from the variable nature of the underlying liver disease, the variable nature of the precipitating events and how they were treated, the heterogeneous and subjective nature of the endpoints, small sample size and short followup.

There are seven randomized control trials dealing with the effect of branched-chain amino acids in acute hepatic encephalopathy. The patients in these trials all had cirrhosis, and GI bleeding and infection were the most common precipitating events. One multicenter trial(82) concluded that patients treated with branched chain amino acids improved more rapidly than patients treated with neomycin; however, the design and results of this trial are so complex as to defy interpretation. In the remaining six (Fig. 7), three compared branch-chain amino acids to nonspecific therapy, ie., glucose or conventional amino acids and no significant differences in recovery from encephalopathy or in mortality were seen(83-85). Three trials compared branched chain amino acids to conventional therapy, ie., lactulose or neomycin and again, no statistically significant differences in recovery from encephalopathy or mortality were observed(86-88). The overall conclusion is that branched-chain amino acids appear to be no better than conventional therapy for acute hepatic encephalopathy. Several clinical reviews and two meta-analyses have been published dealing with these randomized controlled trials and generally agree that current data do not support the use of branched-chain amino acids in acute hepatic encephalopathy(82, 89-91).

Cirrhotics with chronic portal-systemic encephalopathy present different problems then those with acute encephalopathy. Here precipitating events cannot be identified and the patients are, by definition, protein-intolerant and are generally maintained on a low protein diet. However, in patients who may have elevated protein requirements to begin with(92), there is concern that protein restriction may lead to negative nitrogen balance and malnutrition. In turn,

Fig. 7. Randomized controlled trials of BCAA in acute hepatic encephalopathy (Ref. 83-88)

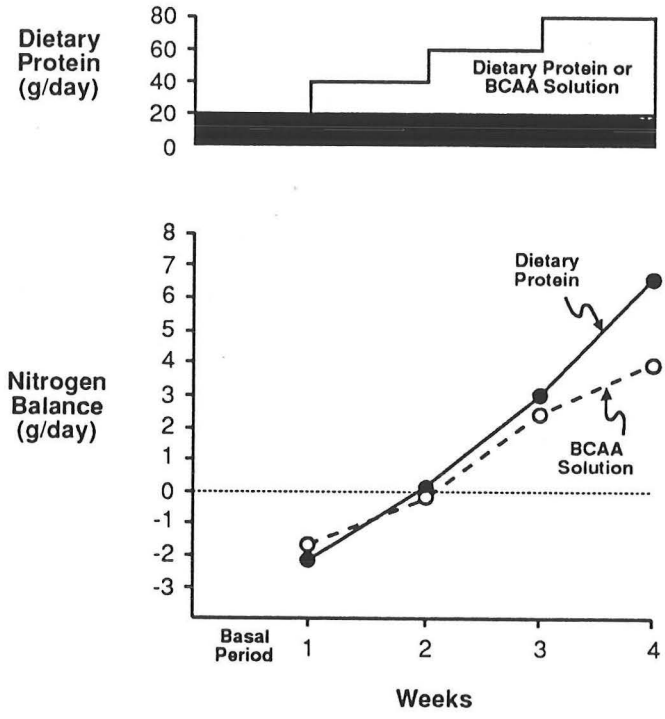


malnutrition and protein breakdown could worsen encephalopathy through decreased peripheral ammonia detoxification and increased amino acid release. Whether branched-chain amino acids can improve mental and/or nutritional status has been examined in at least four randomized controlled trials. Two short-term trials on small numbers of patients showed no advantage of diets enriched with branched-chain amino acid over the control diet(93, 94) whereas a larger multicenter trial suggested an improvement in mental status and nutrition after long-term (3-6 months) supplementation with branched-chain amino acids(95).

A fourth trial compared branched-chain amino acids to regular diet in terms of their encephalopathy-inducing capacity and ability to induce positive nitrogen balance(63). This study involved 26 cirrhotics with chronic portal-systemic encephalopathy who were intolerant of 40 grams of protein per day (Fig. 8). All patients were initially stabilized on a 20 g protein diet. After randomization, the protein content of the diet was increased in increments of 20 g per week up to 80 grams per day using an amino acid solution enriched with branched-chain amino acids or equivalent increments of dietary protein. Nitrogen balance became positive in both groups at about 40 grams of protein per day. However, the mental state deteriorated in seven of the 14 patients in the protein group compared to one in the branched-chain amino acid group. Together these studies suggest that oral branched-chain amino acid supplements

induce positive nitrogen balance to approximately the same degree as an equivalent amount of dietary protein without inducing encephalopathy as frequently. These solutions may be of benefit in patients in whom 40-60 gram protein per day induces recurrent encephalopathy.

Fig. 8. Effect of dietary protein and BCAA solution on nitrogen balance (Ref. 63)



The α-keto analogs of the branched-chain amino acids have also been tried in the treatment of hepatic encephalopathy. The keto analogs can be transaminated to their respective amino acids in the body and thereby may replenish amino acids while simultaneously utilizing ammonia. In one study, the ornithine salts of branched chain ketoacids were found to be more effective than branched chain amino acids in improving the electroencephalographic abnormalities and clinical grade of encephalopathy(96). In contrast, the calcium salts of branched chain ketoacids were not effective(96, 97) nor was ornithine itself. The role of keto analogs in the treatment of hepatic encephalopathy is unresolved.

Benzodiazepine receptor antagonists

Flumazenil (Ro-1788, Anexate, Hoffmann-LaRoche, Basel, Switzerland) is a high-affinity, competitive benzodiazepine receptor antagonist that is available for clinical use in Europe where it is used to reverse the sedative and hypnotic actions of benzodiazepine receptor agonists(98). Following intravenous administration, flumazenil is rapidly cleared from plasma with a $t_{1/2}$ of 45 min. The bioavailability of flumazenil through the oral route is low because of extensive first pass hepatic metabolism. Flumazenil rapidly reverses the sedative and amnestic effects of benzodiazepine agonists administered for anesthesia or diagnostic procedures such as endoscopy(99). Recovery is rapid; however, because of its short half-life, patients who have received long-acting benzodiazepines may require more than one dose. Flumazenil is also

Table III. Flumazenil: efficacy in clinical trials (Ref. 9)

Investigators	No. of cases	No. of responders	Clinical Setting
Bansky et al.	14	10	Cirrhosis
Ferenci and Grimm	1	1	PSE
Pidoux et al.	7	6	Cirrhosis
van der Rijt et al.	17	5	
Klotz and Walker	3	1	Cirrhosis
Grimm et al.	17	10	FHF, Cirrhosis
Burke et al.	1	1	Cirrhosis
James	2	2	FHF
Grimm et al.	6	6	Cirrhosis
Grimm et al.	5	4	FHF, Cirrhosis
Sutherland and Minerk	1	0	FHF
Meier and Gyr	3	3	PSE, Cirrhosis
Bansky et al.	4	2	Cirrhosis
Scollo-Lavizzari and Steinmann	1	1	FHF
Total	**82**	**52 (63%)**	

effective in the treatment of benzodiazepine overdose(100, 101) and in reversing the benzodiazepine component of multidrug overdoses and is useful in terminating the sedative actions of benzodiazepines as a prelude to weaning patients from mechanical ventilators(102).

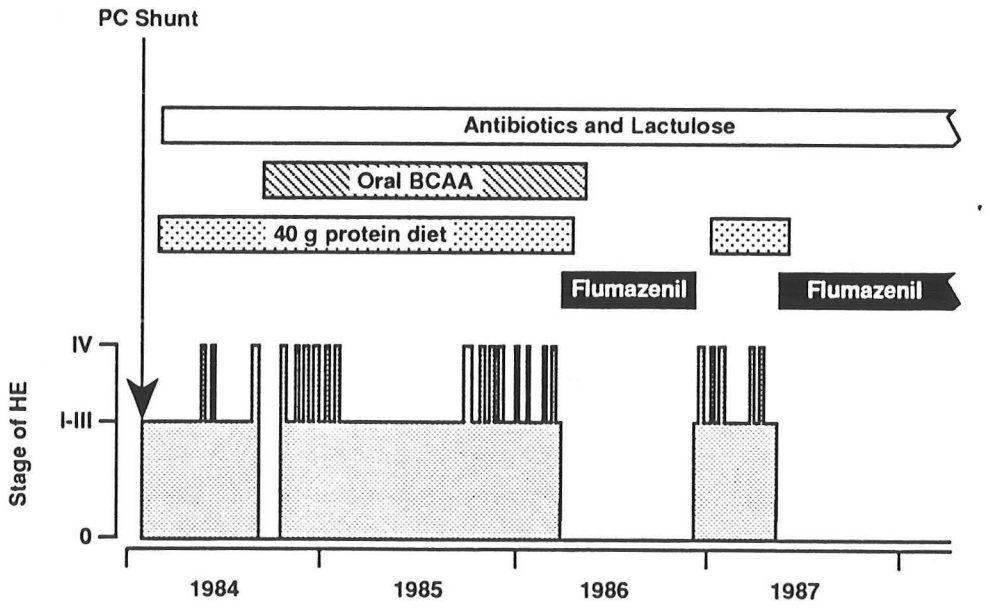
Two anecdotal reports appeared in 1985 reporting the effects of flumazenil in the treatment of hepatic encephalopathy(103, 104). A young woman with fulminant hepatic failure due to hepatitis B was treated with flumazenil. She was in stage IV hepatic encephalopathy, motionless, and unresponsive to painful stimuli and had an EEG showing flat periods alternating with triphasic slow wave complexes. The intravenous administration of 0.5 mg flumazenil improved the EEG and neurologic status such that she opened her eyes, reacted to verbal commands and painful stimuli, and moved spontaneously. The improvement lasted 1 hour and could be sustained with additional flumazenil treatments. The other patient with alcoholic cirrhosis was also in stage IV hepatic encephalopathy. Forty seconds after the intravenous administration of flumazenil (0.3 mg), her encephalopathy had improved to stage II, with responsiveness to verbal and painful stimuli and improvement in her EEG. The improvement lasted about 1 hour and could be regained with additional flumazenil administrations.

Anecdotal observations of improvements in level of consciousness, EEG pattern, and somatosensory-evoked potentials continue to be reported in patients with hepatic encephalopathy who had no known exposure to exogenous benzodiazepines (Table III). In two of the larger studies(105, 106), 19 of 31 patients with acute hepatic encephalopathy responded to

flumazenil administration, with an average improvement of 1.5 hepatic encephalopathy stage units. Compared to conventional treatments for hepatic encephalopathy, the rate of response to flumazenil was rapid, with the time from injection to first response ranging from 28 seconds to a few minutes. The beneficial effects of an intravenous dose of flumazenil were evident for 40 minutes to 4 hour. Nearly all of the patients who did not respond to flumazenil were in stage IV coma and the majority had evidence of increased intracranial pressure and died within 3 days. Indeed, lack of responsiveness to flumazenil may indicate a poor prognosis.

The successful long-term treatment of a patient with chronic intractable portal systemic encephalopathy with orally administered flumazenil was recently described(107). The patient was a 42-year-old woman who underwent a two-thirds hepatectomy and an end-to-side portacaval shunt (Fig. 9). Three weeks after surgery the patient became encephalopathic. The encephalopathy was inadequately controlled by dietary protein restriction, lactulose, and antibiotics. During a period of several months, she fluctuated between stage I-III hepatic encephalopathy and was hospitalized multiple times in hepatic coma. During an episode of deep coma, the patient regained consciousness 30 seconds after the administration of 1 mg flumazenil and remained conscious for 2 hours. Because of this favorable response, oral flumazenil (25 mg b.i.d.) was initiated, and a sustained reversal of the encephalopathy was achieved despite protein

Fig. 9. Remissions of chronic intractable PSE with the oral administration of flumazenil (Ref. 107)



intakes up to 150 gram per day. During long-term flumazenil treatment there was no clinical evidence of even mild hepatic encephalopathy and the results of several sensitive tests for hepatic encephalopathy such as psychometric tests and serial multimodality evoked potential recordings were normal. The flumazenil was well-tolerated although she consistently became anxious about 25-40 min after the intake of the drug and remained so for 30-60 min. After 9 months, flumazenil was discontinued as part of an assessment of fever of unknown origin. Two days later she became comatose and remained encephalopathic with episodes of coma until flumazenil was restarted, after which, she again became free of signs of hepatic encephalopathy. This case raises the possibility that benzodiazepine receptor antagonists may be safe and effective in the long-term treatment of chronic portal-systemic encephalopathy.

Benzodiazepine receptor antagonists appear to have several potential applications in the management of hepatic encephalopathy(108). First, they can be used to reverse the effects of exogenously administered benzodiazepines. Second, the patients response to a benzodiazepine receptor antagonist may be useful in the differential diagnosis of encephalopathies or as an index of prognosis. A favorable response may indicate that encephalopathy is uncomplicated, and potentially reversible. Third, these drugs may be administered, perhaps by intravenous infusion, in an attempt to optimize brain function in patients with acute hepatic encephalopathy. Finally, when given orally, these drugs may improve protein tolerance in patients with chronic portal-systemic encephalopathy.

Miscellaneous therapies

In some patients, severe encephalopathy may develop following a portacaval shunt for variceal bleeding. In these patients, encephalopathy can be reversed by shunt occlusion. This may be preceded by an esophageal transection to avoid the risk of re-bleeding(109) or by balloon occlusion.

The false neurotransmitter hypothesis suggests that catecholamine mediated neurotransmission may be reduced in hepatic encephalopathy. This led to the trial of dopaminergic agents such as L-DOPA and bromocriptine, both of which have been shown to be ineffective in randomized controlled trials(110, 111).

Finally, liver transplantation is becoming an increasingly important option for patients with decompensated chronic liver failure or fulminant hepatic failure(112). As expected, when the failing liver is successfully replaced by a normally functioning liver, complete and sustained resolution of hepatic encephalopathy occurs.

Conclusion

Clearly the mechanisms responsible for hepatic encephalopathy are unresolved. Because of the host of metabolic abnormalities that accompany liver failure, it has been difficult to sort out those factors that are causally related from those that are epiphenomena. Nevertheless, the genesis of hepatic encephalopathy appears to involve the accumulation of neuroactive and potentially comagenic substances in the systemic circulation that are derived from the gut and normally cleared and metabolized by the liver. Standard therapy for hepatic encephalopathy is directed at identifying and correcting any precipitating factors and reducing the absorption of nitrogenous substances from the gut. Recently, evidence has accumulated suggesting that alterations in GABAergic neurotransmission secondary to increased levels of benzodiazepine receptor ligands may contribute to brain dysfunction in many patients with hepatic encephalopathy. Currently available data (which are largely anecdotal) suggest that benzodiazepine receptor antagonists are likely to be an important adjunct to conventional therapies for hepatic encephalopathy.

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