Clinical and experimental aspects of hepatic encephalopathy

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Klinische en experimentele aspecten van hepatische encephalopathie

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Indoor fireworks
Can still burn your fingers
Indoor fireworks
We swore we were as safe as houses
They are not so spectacular
They do not burn up in the sky
But they can dazzle or delight
Or bring a tear
When the smoke gets in your eyes

Elvis Costello: Indoor Fireworks
Van het album: "King of America" (CBS Inc., 1986)

Voor Isis en Ernő

LIST OF ABBREVIATIONS

ALF	acute liver failure
AMM	hyperammonemia
AU	arbitrary units
AUC	area under curve
CPS	carbamyl phosphate synthetase
CSF	cerebrospinal fluid
CON	controls
D1	type 1 deiodinase
DST	Digit Symbol Test
EEG	electroencephalogram
FT_4	free thyroxin
GABA	γ aminobutyric acid
GFAP	glial fibrillary acidic protein
HE	hepatic encephalopathy
MDF	mean dominant frequency
MRI	magnetic resonance imaging
NCT-A	Number Connection Test part A
NF-68	neurofilament 68
NF-200	neurofilament 200
NMDA	N-methyl-D-aspartate
NSE	neuron specific enolase
NTI	non-thyroidal illness
OTC	ornithine transcarbamylase
PBC	primary biliary cirrhosis
PCS	portacaval shunt
PSE	portal-systemic encephalopathy
ROC	receiver operating characteristic
rT_3	reverse triiodothyronine
SEM	standard error of mean
SHE	subclinical hepatic encephalopathy
SIP	Sickness Impact Profile
TIPS	Transjugular Intrahepatic Portal-systemic Shunt
T_3	triiodothyronine
T_4	thyroxine
T_3S	triiodothyronine sulphate
T_4S	thyroxine sulphate

CONTENTS

Introduction

The syndrome of hepatic encephalopathy

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome associated with severe liver disease. Clinical symptoms range from minimal changes in mental state and neuromuscular defects to unresponsive coma. [1-3] The syndrome of HE can be divided into three major groups: HE associated with liver cirrhosis, HE associated with acute liver necrosis and HE associated with errors of metabolism (table 1).

HE is most often observed in patients with cirrhosis of the liver, either as a result of decreased hepatic parenchymal function or as a result of increasing portal-systemic shunting without liver insufficiency (also defined as portal-systemic encephalopathy [1-3]). In patients with cirrhosis, HE can be induced or aggravated by a number of precipitating factors (table 2). [4]

HE associated with acute liver necrosis has a dramatic clinical course, leading to acute liver failure in most patients. Apart from jaundice, HE can be the first alarming symptom of acute liver failure. The degree of HE is a strong predictor of outcome in acute liver failure; patients who only reach grade II HE have a possibility of spontaneous recovery of 65 - 70 %; with grade III HE it is 40 - 50 % and in grade IV HE it is only 20 %. In the majority of patients with grade III HE cerebral edema is present, which can evolve to an increase in intracranial pressure, cerebral herniation and death. [5]

Table 1 Hepatic encephalopathy: the syndromes

HE	⇒	liver cirrhosis	1. decreased hepatic parenchymal function 2. increased portal-systemic shunting 3. precipitating factors (table 2)
HE	⇒	acute liver necrosis	
HE	⇒	errors of metabolism	1. urea cycle enzyme deficiency (OTC, CPS) 2. organic acidemias 3. Reye's syndrome (secondary urea cycle deficiency and liver necrosis)

HE associated with errors of metabolism includes inborn errors, like: urea cycle enzyme defects (predominantly carbamyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC)) and organic acidemias. In addition, Reye's syndrome in children results in secondary urea cycle enzyme deficiency, fatty infiltration of the liver and liver necrosis.[6-8] Reye's syndrome is characterised by progressive HE in a previously healthy child. Classically, this syndrome has a biphasic course, in which progressive HE develops after a prodromal febrile illness (an upper respiratory tract infection or chickenpox) and an interval of 5 - 7 days without symptoms. [12-13]

HE associated with acute liver necrosis, urea cycle enzyme deficiencies and Reye's syndrome can have a rapidly progressive course, and survival of patients is dependent on the degree of HE and cerebral edema. Symptomatic treatment of cerebral edema and hyperammonemia often fails to prevent cerebral herniation, structural brain damage and death. Although liver transplantation is urgently indicated in patients with progressive cerebral complications, a suitable donor liver is often not available in time. The understanding of the mechanism of HE and cerebral edema formation could help to develop therapeutic strategies specifically directed to these cerebral complications. Therefore, in this thesis there is a focus on the pathophysiology of HE.
Of all syndromes of HE, the syndrome of HE associated with liver cirrhosis is most prevalent. HE in cirrhosis is often the result of decreased hepatic parenchymal damage or increased portal-systemic shunting. However, in about 60 to 70 % of cases one or more precipitating factor can be identified (table 2). HE in cirrhosis can be treated satisfactory, either by dietary protein restriction, lactulose, antibiotics, or sodium benzoate, or by eliminating one or more precipitating factors. As a last therapeutic option, liver transplantation is available to cirrhotic patients with liver insufficiency and recurrent episodes of HE. [4,9-11]

However, two major groups of patients with cirrhosis can be identified, which have an Increased risk of chronic or recurrent episodes of HE, and which cannot be satisfactory treated: Firstly, patients with contra indications for liver transplantation (e.g. old cirrhotic patients and patients treated with a Transjugular Intrahepatic Portosystemic Shunt (TIPS)); Secondly, patients with neuropsychological or neurophysiological defects, without clinical signs of HE, also defined as subclinical hepatic encephalopathy (SHE). [12] In this thesis we focussed on the syndrome of SHE, because its diagnostic criteria, its clinical implication, and its effect on daily functioning is largely unknown.

Table 2 Factors precipitating hepatic encephalopathy in cirrhosis [4]

precipitating factor	possible mechanism
- Excess dietary protein - Constipation - Gastrointestinal haemorrhage - Infection / bacterial peritonitis - Blood transfusion - Azotemia - Hypokalemia	Increased ammonia production
- Systemic alkalosis	Increased diffusion of ammonia across blood-brain-barrier
- Dehydration (fluid restriction, diuretics, excessive paracentesis, diarrhea due to osmotic laxatives) - Arterial hypotension (gastrointestinal haemorrhage, peripheral vascular dilatation) - Anaemia	Reduced metabolism of toxins because of hepatic hypoxia
- Use of benzodiazepines	Activation of GABA-benzodiazepine receptors
- Use of other psychoactive drugs	CNS depressant effect
- Portosystemic shunts (spontaneous, surgically, Transjugular Intrahepatic Portosystemic Shunt (TIPS))	Reduced hepatic metabolism of toxins because of diversion of portal blood
- Progressive hepatic parenchymal damage - Hepatocellular carcinoma	Reduced hepatic metabolism of toxins because of decreased functional reserve

PATHOPHYSIOLOGICAL ASPECTS OF HEPATIC ENCEPHALOPATHY

Ammonia and glutamate

Ammonia is still the leading factor considered to have a causative relation with regard to HE. [13] There are three factors that support a causative relation[14] : Firstly, ammonia is usually elevated in patients with HE; [15, 16] in patients with other hyperammonemia syndromes, like urea cycle enzyme deficiencies, organic acidemias and in neonates with transient hyperammonemia, hyperammonemia is accompanied by encephalopathy; [6 - 8] Secondly, experimentally induced hyperammonemia induces signs of encephalopathy; [17, 18] Thirdly, treatment directed to lowering blood ammonia levels - using either protein restriction, lactulose, oral antibiotics or sodium benzoate - reverse signs of HE. [14, 16]

The mechanism of ammonia toxicity is still largely unknown. In essence, ammonia affects brain metabolism in three ways: either by interference with cell metabolism, with electrophysiologic membrane function, or with neurotransmitter function. [13, 19, 20] The interference of ammonia with neurotransmitter function is of interest, because it is still unknown whether ammonia itself or ammonia-related amino acids - like glutamate and glutamine - are the toxic factor. Previous studies at our department on the effect of ammonia on brain neurotransmitter function has led to the hypothesis, that glutamate neurotoxicity may be related to structural brain damage and to HE. [27, 45]

Glutamate is the principal excitatory neurotransmitter in the mammalian brain, [21, 22] and there is substantial evidence showing its involvement in a large number of neurological diseases, like epilepsy, Alzheimer disease and in stroke-induced brain damage. [21 - 23] The following experimental data also support the involvement of glutamate in HE: Firstly, total brain glutamate levels are significantly reduced in autopsied brain tissue of patients with acute and chronic liver disease who died in hepatic coma, [24, 25] suggesting altered glutamate metabolic pathways between neurons and glial cells; [26] Secondly, extracellular concentrations of glutamate are increased significantly in portacaval shunted rats, [27] and in rabbits with acute hyperammonemia and with acute liver failure, [25] probably as a result of an ammonia-induced decrease in glutamate re-uptake into astrocytes. [26] Increased post-synaptic stimulation by glutamate down-regulates non-NMDA-glutaminergic receptors (kainate and AMPA) in experimental acute liver failure, [28] which results in a relative over-exposure of the NMDA receptor. Over-exposure of the NMDA receptor leads to increased calcium influx intracellularly, disturbance of ion homeostasis, and cellular swelling. Eventually this neurotoxicity leads to cell death. [23]

The GABA-benzodiazepine receptor complex

γ-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the mammalian brain, and it comprise 30 % of the total neurotransmitter content. [29, 30] Binding of benzodiazepines to the GABA-benzodiazepine receptor complex potentiates the inhibitory effect of GABA. [29] Shafer and Jones [31] were the first to postulate, that GABA synthesized by intestinal bacteria escaping hepatic detoxicification, could induce signs of HE. Initial studies on this issue supported this concept: Firstly, increased GABA-like activity was found in serum of patients with HE; [32] Secondly, enhanced transfer of α-aminobutyric acid and GABA across the blood brain barrier was found in rabbits with acute liver failure, [33] and thirdly: GABA receptors were down-regulated in brains of patients with HE. [34] However, these findings could not be confirmed by others. [12] Therefore, attention shifted to the concept of endogenous benzodiazepine activity, stating that HE may be induced by the accumulation of benzodiazepine-like substances in the blood. [35] Although increased "endogenous" benzodiazepine activity was found in patients with HE, [36] results may have been biased by exogenous benzodiazepine use and by benzodiazepine-

like substances in the diet. The ultimate test to evaluate the role of benzodiazepines in HE is the application of a benzodiazepine antagonist. However, controlled trials on this issue in patients with HE show conflicting results. [37-40] The unresolved issue of the GABA-benzodiazepine made us decide to re-evaluate the efficacy of the benzodiazepine antagonist flumazenil at our department, using objective criteria.

Thyroid hormone metabolism

Altered thyroid hormone metabolism is a common finding in patients with chronic liver disease. [41, 42] Low triiodothyronine (T_3) and low thyroxine (T_4) levels have been described, without clinical signs of hypothyroidism. In general, central regulation of the thyroid gland is preserved in patients with chronic liver disease. The observed changes in thyroid hormone metabolism have therefore been classified as non-thyroidal illness (NTI). [43, 44] A case-control study performed at our department indicated, that serum total T_3 has strong independent predictive power for HE. [45] In addition, a recent case-report suggested, that supplementation of thyroid hormone may improve symptoms of HE. [46] We therefore hypothesize, that altered thyroid hormone metabolism has a role in the pathogenesis of hepatic encephalopathy.

Other potential fields in the pathophysiology of HE

Tryptophan

Tryptophan was found to induce HE in Eck fisula dogs. [47] In cirrhotic patients with HE, tryptophan is increased, compared to patients without HE. [48] Tryptophan is a precursor of serotonin (5-HT), and increased serotonin turnover was observed in brains of rats with a portacaval shunt and ammonia infusion. [49] More recently, studies in brain tissue from cirrhotic patients who died in hepatic coma revealed increased activity of the serotonin-degrading enzyme monoamine oxidase. [50] In addition, increased densities of post-synaptic serotonin binding sites ($5HT_2$) were found. These findings indicate, that serotonin metabolism is increased in HE, probably accompanied by a serotonergic synaptic deficit. [51] The exact mechanism by which tryptophan could contribute to HE is under investigation, with focus on the kynurenic pathway. Through the kynurenine pathway L-kynurenine is produced out of tryptophan. L-kynurenine can modulate excitatory neurotransmission; in addition, from L-kynurenine, quinolinic acid is formed. This substance has very potent excitatory properties and it could induce excitotoxic damage to the brain. [52,53] The following experimental data confirm the possible role of quinolinic acid in HE: In rats with a portacaval shunt whole brain quinolinic acid was increased. [54] In cerebrospinal fluid of patients who died in HE quinolinic acid was 3-fold increased, compared to control subjects. [55] In children with congenital hyperammonemia a 2 - 10 - fold increase in CSF quinolinic acid was found; the increase was significantly higher in neonates in hyperammonemic coma, compared to older children without coma. [56]

Manganese

Pallidal hyperintensity on T1-weighted MRI is a confirmed finding in patients with cirrhosis [57, 58], which may be related to manganese accumulation. [59] Analysis of blood samples of cirrhotic patients showed significantly increased manganese levels, as compared to controls, and manganese levels were correlated with the degree of pallidal hyperintensity.[60] The role of manganese accumulation in the pathogenesis of HE - and especially its effect on dopaminergic neurotransmission in basal ganglia - remains undefined.

Glutamine and myo-inositol

Brain ammonia is detoxified in astrocytes by glutamine synthetase. In experimental hyperammonemia, brain glutamine levels are significantly increased [18, 25-27], and in patients with acute liver failure, a 6-fold elevation of glutamine was observed. [61] Glutamine accumulates in astrocytes, which could result in astrocytic swelling and the formation of brain edema. Inhibition of glutamine synthesis by methionine-sulfoximine prevents brain swelling in rats after portacaval anastomosis. [62-64] In patients with cirrhosis, HE is rarely associated with brain edema formation, suggesting that the osmotic effect of glutamine accumulation is compensated by osmoregulatory mechanisms. [65] Studies on the organic osmolite myo-inositol in patients with chronic HE showed, that myo-inositol is decreased intracellularly (measured by NMR spectroscopy), indicating that astrocytes try to preserve their osmotic balance by extruding myo-inositol.[66] In addition, in rats with a portacaval shunt, a time-dependent change in organic brain osmolytes was observed. [67] The implications of these osmoregulatory changes for the pathophysiology of HE is still undefined.

SUBCLINICAL HEPATIC ENCEPHALOPATHY

Hepatic encephalopathy is tradionally graded into four grades, ranging from subtle mental impairment to unresponsive coma. The classical clinical grading system is presented in table 3a. We have had considerable difficulties in defining the various items. The application of this system by two experienced clinicians resulted in discrepancies in at least one third of patients. [45]

Table 3a Clinical grading of hepatic encephalopathy

Grade 0	No abnormality
Grade 1	Trivial lack of awareness
	Euphoria or anxiety
	Shortened attention span
	Impaired performance of addition
Grade 2	Lethargy or apathy
	Minimal disorientation of time or place
	Subtle personality change
	Inappropriate behaviour
	Impaired performance of subtraction
Grade 3	Somnolence to semi-stupor, but responsive to verbal stimuli
	Confusion
	Gross disorientation
Grade 4	Coma (unresponsive to verbal or noxious stimuli)

Table 3b Adapted clinical grading of hepatic encephalopathy

Grade 0	**absence of at least three of the following abnormalities:** - inverted sleep pattern - disturbed memory - impaired calculation - slowness of speech
Grade 1	**presence of at least two of these abnormalities, or one abnormality in association with a flapping tremor**
Grade 2	**presence of at least two of the following abnormalities:** - lethargy - disorientation in time - flapping tremor
Grade 3	**presence of at least two of the following abnormalities:** - a state in which the patient must be urged repeatedly to open his eyes or execute a command - disorientation in place - disorientation in person
Grade 4	**coma**

Therefore, we now use an in-house grading system, aiming at a more reproducible classification of patients, and based on the classical four stages (table 3b).
Still, clinical grading lacks precision and is subject to considerable inter-observer variability. [12] In addition, the difference between grade 0 ("no abnormality") and grade 1 ("trivial lack of awareness") is poorly defined; the adapted grading system by Van der Rijt does not solve this problem. Most patients with cirrhosis either have grade 0 or grade 1 HE. However, a considerable number of patients without clinical HE (grade 0) have subtle deficits in neuropsychiatric functions, using neuropsychological and neurophysiological tests. [68-70] This abnormality has been defined as subclinical hepatic encephalopathy (SHE). [71]
The prevalence of SHE varies between 30 - 84% in patients with liver cirrhosis, dependent on the diagnostic criteria used.[68-70] At our department, studies on the diagnosis of SHE have been performed, using psychometric tests (Number Connection test A and Digit Symbol Test), and automated EEG analysis. SHE is defined present in a patient without clinical signs of HE, if at least one psychometric test is abnormal and/or if there is abnormal slowing of the EEG.[71-72] The prevalence of SHE as diagnosed by these criteria is about 23 %. Presence of SHE is correlated with the severity of liver disease, ranging from 14 % of patients with Child-Pugh A to 45 % of patients with Child Pugh B/C. In addition, older patients with cirrhosis with higher arterial ammonia levels have an increased risk to develop SHE, compared to younger patients. [71]
The significance of the diagnosis SHE is subject to debate. [72, 73] Recent studies show, that SHE has a negative influence on daily functioning. [74, 75] In addition, several investigators suggested, that there is a relation between SHE and the subsequent development of episodes of clinical hepatic encephalopathy. [12, 76] However, studies on the natural history of SHE and its prognosis with respect to development of clinical hepatic encephalopathy and survival are lacking. [12, 72]
To limit the inter-observer variability of clinical grading, automated EEG analysis has been developed at our department. [77] This method is now standard procedure in the evaluation of HE in our clinic, and it is used for the diagnosis of SHE.
Studies have indicated, that the EEG of patients with clinical HE can vary during the day, and especially after a meal. [78] To be able to use automated EEG analysis as an optimal diagnostic test, data on its variability during the day should be available.

Focus of research on hepatic encephalopathy in 1994

In summary, in 1994, when protocols were written that form the basis of the studies included in this thesis, our research on the pathophysiology of HE primarily focussed on ammonia-induced alterations in glutamate neurotransmission, structural brain damage (neuronal and glial marker proteins), the GABA-benzodiazepine receptor complex (flumazenil) and thyroid hormone metabolism. Four studies on the pathophysiology addressing some of these subjects, are included in this thesis.
Our clinical research on HE primarily focussed on the diagnosis and implications of subclinical hepatic encephalopathy (SHE). Four studies on SHE are included in this thesis.

AIMS OF THE THESIS

Studies on the pathophysiology

- To evaluate structural changes in the brain of rabbits with encephalopathy associated with acute liver failure and acute hyperammonemia (chapter 1.1)
- To study the effect of the glutamate receptor antagonist MK-801 on encephalopathy and survival of rabbits with encephalopathy associated with acute liver failure and acute hyperammonemia (chapter 1.2)
- To study the role of thyroid hormone metabolism in the pathophysiology of hepatic encephalopathy (chapter 1.3)
- To study the clinical and electrophysiological effect of flumazenil in patients with hepatic encephalopathy (chapter 1.4)

Studies on subclinical hepatic encephalopathy

- To study the variability of automated EEG analysis during the day in patients with subclinical and clinical hepatic encephalopathy (chapter 2.1)
- To study the effect of subclinical hepatic encephalopathy on daily functioning (chapter 2.2)
- To study the diagnostic applicability of symptoms specifically related to subclinical hepatic encephalopathy (chapter 2.3)
- To study the prognostic significance of subclinical hepatic encephalopathy (chapter 2.4)

References

1. Sherlock S, Summerskill WHJ, White LP, Phear EA. Portal systemic encephalopathy: neurological complications of liver disease. Lancet 1954;2:453-57.
2. Conn HO. Quantifying the severity of hepatic encephalopathy. In: Conn HO, Bircher J, editors. Hepatic encephalopathy: syndromes and therapies. Bloomington: Medi-Ed press; 1994. p. 13-26.
3. Lockwood AH. Clinical and laboratory features of hepatic encephalopathy.In: Lockwood AH editor. Hepatic encephalopathy. Butterworth-Heinemann, Stoneham, MA, USA, 1992. p. 11-32.
4. Riordan SM, Williams R. Treatment of hepatic encephalopathy (review). New Eng J Med 1997;337:473-79.
5. Hoofnagle JH, Carithers RL, Shapiro C, Ascher N. Fluminant hepatic failure: summary of a workshop (special article). Hepatology 1995;21:240-52.
6. Greene CK, Blitzer MF, Shapira E. Inborn errors of metabolism and Reye's syndrome: differential diagnosis. J Pediatr 1988;113:156.
7. Rosenthal P, Vinocur B. Neonatal hyperammonemia. J Pediatr 1979;94:847-48.
8. Ballard RA, Vinocur B, Reynolds JW. Transient hyperammonemia of the preterm infant. New Engl J Med 1978;299:920-35.
9. Ginés P, Quitero E, Arroyo V, Ters J, Bruguera M, Rimola A, Caballeria J, Rodes J, Rozman C. Compensated cirrhosis: natural history and prgnostic factors. Hepatology 1987;7:122-8.
10. Powell EE, Pender MP, Chalk JB. Improvement in chronic hepatocerebral degeneration following liver transplantation. Gastroenterology 1990;98:1079-82.
11. Conn HO. The encephalopathies. In : Conn HO, Bircher J. eds. Hepatic encephalopathy: management with lactulose and related carbohydrates. Medi-Ed Press, East Lansing, Michigan, USA 1988:3-14.
12. Mullen KD, Gacad R. Hepatic encephalopathy. The Gastroenterologist 1996;4:188-202.
13. Butterworth RF. Pathophysiology of hepatic encephalopathy; the ammonia hypothesis revisited. In: Hepatic encephalopathy and metabolic nitrogen exchange. Bengtsson F, Jeppsson B, Almdal T, Vilstrup H eds. CRC Press, Boca Raton, 1991, 9-24.
14. Zieve L. The mechanism of hepatic coma. Hepatology 1981;1:360-65.
15. Phear EA, Sherlock S, Summerskill WHJ. Blood ammonium levels in liver disease and hepatic coma. Lancet 1955;1:836-40.
16. Treager HS ,Gabuzda SJ, Ballou AN, Davidson CS. Blood ammonia concentration in liver disease and liver coma. Metabolism 1954;3:99-109.
17. De Knegt RJ, Groeneweg M, Schalm SW, Hekking-Weijma I. Encephalopathy from acute liver failure and from acute hyperammonemia in the rabbit. A Clinical and biochemical study. Liver 1994;14:25-31.
18. Bosman K , Deutz NEP, De Graaf AA, Van de Hulst RWN, Van Eijk HMH, Bovee WMMJ, Maas MAW , Jörning GGA, Chamuleau RAFM. Changes in brain metabolism during hyperammonemia and acute liver failure: results of a comparative ^{1}H-NMR spectrosocpy and biochemical investigation. Hepatology 1990;12:281-90.
19. Raabe W. Synaptic transmission in ammonia intoxication. Neurochem Pathol 1987;6:145-66.
20. Norenberg MD. The astrocyte in liver disease. In Federoff S and Hertz L. (eds.) Advances in cellular biology 2. New York Academic Press, 1981, pp. 303-52.
21. Fonnum F. Glutamate: a neurotransmitter in mammalian brain. J Neurochem 1984;42:1-11.
22. Butterworth RF. Evidence that hepatic encephalopathy results from a defect of glutamatergic synaptic regulation. Mol Neuropharmacol 1992;2:229-32.
23. Choi DW. Ionic dependence of glutamate neurotoxicity. J Neurosci 1987;7: 369-79.
24. Lavoie J, Giguère J-F, Pomier Layrargues G, Butterworth RF. Amino acid changes in autopsied brain tissue

from cirrhotic patients with hepatic encephalopathy. J Neurochem 1987;49:692-97.

25. Tossman U, Delin A, Eriksson LS, Ungerstedt U. Brain cortical amino acids measured by intracerebral dialysis in portacaval shunted rats. Neurochem Res 1987;12:265.
26. Butterworth RF. Hepatic encephalopathy and brain edema in acute hepatic failure: does glutamate play a role ? (editorial). Hepatology 1997;25:1032-34.
27. De Knegt RJ, Schalm SW, van der Rijt CCD, Fekkes D, Daim E, Hekking I. Extracellular brain glutamate during acute liver failure and during acute hyperammonemia simulating acute hepatic failure: an experimental study based on in vivo brain dialysis. J Hepatol 1994; 20: 119-26.
28. Michalak M, Butterworth RF. Selective loss of binding sites for the glutamate receptor ligand [^{3}H]5-fluorowillardiine in the brains of rats with acute liver failure. Hepatology 1997;25:631-35.
29. Walker JE. Glutamate, GABA and CNS disease: a review. Neurochem Res 1983;8:521-50.
30. Ticku MK. Benzodiazepine-GABA receptor ionophore complex. Current concepts. Neuropharmacology 1983;22:1459-70.
31. Schafer DF, Jones EA. Hepatic encephalopathy and the y-aminobutyric-acid neurotransmitter system. Lancet 1982;1:18-20.
32. Ferenci P, Kleinberger G, Schafer DF, Hoofnagle JH, Jones EA. Serum levels of gamma-aminobutyric-acid-like activity in acute and chronic hepatocellular disease. Lancet 1983:811-14.
33. Bassett ML, Mullen KD, Scholz B, Fenstermacher JD, Jones EA. Increased brain uptake of gamma-aminobutyric acid in a rabbit model of hepatic encephalopathy. Gastroenterology 1990;98:747-57.
34. Schafer DF, Fowler JM, Munson PJ, Thakur AK, Waggoner JG, Jones EA. Gamma-amminobutyric acid (GABA) in patients with hepatic encephalopathy by the GABA-radioreceptor assay. Hepatology 1988;8:69.
35. Mullen KD, Martin JV, Mendelson WB. Could an endogenous benzodiazepine ligand contribute to hepatic encephalopathy ? Lancet 1981;1:457-59.
36. Mullen KD, Szauter KM, Kaminski-Russ K. "Endogenous" benzodiazepine activity in body fluids of patients with hepatic encephalopathy. Lancet 1990;1:81-3.
37. Pomier-Layrargues G, Giguère, Lavoie J, Perney P, Gagnon S, D'Amour M, Wells J and Butterworth RF. Flumazenil in cirrhotic patients in hepatic coma: a randomized double-blind placebo-controlled crossover trial. Hepatology 1994;19:32-7.
38. Cadranel JF, EL Younsi M, Pidoux B, Zylberberg P, Benhamou Y, Valla D, Opolon P. Immediate improvement of hepatic encephalopathy (HE) in cirrhotic patients by flumazenil. Results of a double-blind cross-over study. J Hepatol 1991;13 Suppl 13:S104.
39. Van der Rijt CCD, Schalm SW, Meulstee J, Stijnen Th. Flumazenil therapy for hepatic encephalopathy: a double-blind cross-over study. Hepatology 1989;10:A85.
40. Gyr K, Meier R, Häussler, Boulétreau P, Fleig WE, Gatta A et al. Evaluation of the efficacy and safety of flumazenil in the treatment of portal systemic encephalopathy: a double blind, randomised, placebo controlled multicentre study. Gut 1996;39:319-24.
41. Kaptein EM. Thyroid hormone metabolism in illness. In: Hennemann G ed. Thyroid hormone metabolism, basic and clinical endocrinology series, volume 8, New York, USA, Marcel-Dekker, 1986:297-333.
42. Sheridon P. Thyroid hormones and the liver. Clinics in Gastroenterology 1983;12:797-818.
43. Green JRB, Snitcher EJ, Mowat NAG et al. Thyroid function and thyroid regulation in euthyroid men with chronic liver disease: evidence of multiple abnormalities. Clin Endocrinol 1977;7:453-61.
44. Chopra IJ, Hersman JM, Pardridge WM, Nicoloff JT. Thyroid function in nonthyroidal illnesses (UCLA conference). Ann Int Med 1983;98:946-957.
45. Rijt CCD van der. Hepatic encephalopathy: clinical and experimental studies [dissertation]. Rotterdam:Erasmus Univ.;1991.

46. Hitoshi S, Terao Y, Sakuta M. Portal-systemic encephalopathy and hypothalamic hypothyroidism: effect of thyroid hormone on ammonia metabolism (case report). Int Med 1993;32:655-658.
47. Ogihara K, Mozai T, Hirai S. Tryptophan as a cause of hepatic coma. New Eng J Med 1966;275(22):1255-
48. Ono J, Hutson DG, Dombro RS, Levi JU, Livingstone A, Zeppa R. Tryptophan in hepatic coma. Gastroenterol 1978;74:196-200.
49. Bergeron M, Swain MS, Reader TA, Grondin L, Butterworth RF. Effect of ammonia on brain serotonin metabolism in relation to function in the portacaval shunted rat. J Neurochem 1990;55:222-229.
50. Rao VLR, Agrawal AK, Murthy CRK. Ammonia-induced alterations in glutamate and muscimol binding to cerebellar synaptic membranes. Neurosci Lett 1991;130:251-54.
51. Butterworth RF. Hepatic encephalopathy: disorder of multiple neurotransmitter systems. In: Record CO, Al Mardini H editors. Advances in hepatic encephalopathy and metabolism in liver disease. Ipswich, UK: Ipswich Book Company; 1997. P. 167-75.
52. Stone TW. Neuropharmacology of quinolinic and kynurenic acids. Pharmacol Rev 1993;45(3):309-379.
53. Schwarcs R, Du F, Schmidt W, Turski WA, Gramsbergen JBP, Okuno E, Roberts RC. Kynurenic acid: a potential pathogen in brain disorders. Ann NY Acad Sci 1992;648:140-153. 47.
54. Moroni F, Lombardi G, Carlà V, Pellegrini D, Carassale GL, Cortesini C. Content of quinolinic acid and of other tryptophan metabolites increases in brain regions of rats using as experimental models of hepatic encephalopathy. J Neurochem 1986;46:869-874.
55. Moroni F, Lombardi G, Carlà V, Lal S, Etienne P, Nair NPV. Increase in the content of quinolinic acid in cerebrospinal fluid and frontal cortex of patients with hepatic failure. J Neurochem 1986;47:1667-1671.
56. Batshaw ML, Robinson MB, Hyland K, Djali S, Heyes MP. Quinolinic acid in children with congenital hyperammonemia. Ann Neurol 1993;34(5):676-681.
57. Pujol A, Pujol J, Graus F, Rimola A, Peri J, Mercader JM, Garcia-Pagan JC, Bosch J, Rodes J, Tolosa E. Hyperintense globus pallidus on T1-weighted MRI in cirrhotic patients is associated with severity of liver failure. Neurology 1993;43:65-69.
58. Weissenborn K, Ehren HE, Hori A, Kubicka S, Manns MP. Pallidal lesions in patients with liver cirrhosis: clinical and MRI evaluation. Metab Brain Dis 1995;10:219-28.
59. Butterworth RF, Spahr L, Fontaine S, Pomier-Layrargues G. Managese toxicity, dopaminergic dysfunction and hepatic encephalopathy. Metab Brain Dis 1995;10:259-67.
60. Spahr L, Butterworth RF, Fontaine S, Therrien G, Milette PC, Lebrun LH, Zayed J, Leblanc A, Pomier-Layrargues G. Increased blood manganese in cirrhotic patients: relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms. Hepatology 1996;24:1116-1120.
61. Record CO, Buxton B, Chase RA, Curzon G, Murray-Lyon IM , Williams R. Plasma and brain aminoacids in fulminant hepatic failure and their relationship to hepatic encephalopathy. Eur J Clin Invest 1976;6:387-94.
62. Takahashi H, Koehler RC, Brusilow SW, Traystman RJ. Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. Am J Physiol 1991;261:H825-29.
63. Blei AT, Olafsson S, Therrien G, Butterworth RF. Ammonia-induced brain edema and intracranial hypertension in rats after portacaval anastomosis. Hepatology 1994;19:1431-44.
64. Norenberg MD, Bender AS. Astrocyte swelling in liver failure: role of glutamine and benzodiazepines. Aca Neurochir 1994;60 (Suppl):24-27.
65. Blei AT. Brain edema - Its relation to hepatic encephalopathy. In: Record CO, Al Mardini H editors. Advances in hepatic encephalopathy and metabolism in liver disease. Ipswich, UK: Ipswich Book Comp; 1997: P. 259.
66. Haussinger D, Laubenberger J, Von Dahl S, Ernst T, Bayer S, Langer M, Gerok W, Hennig J. Proton magnetic resonance spectroscpoy studies on human brain myo-inositol on hypoosmolarity and hepatic encephalopathy. Gastroenterology 1994;1475-80.

67. Cordoba J, Gottstein J, Blei AT. Glutamine, myo-inositol and organic osmolytes in rats after portacaval anastomosis. Implications for ammonia-induced brain edema. Hepatology 1996.
68. Zeegen R, Drinkwater JE, Dawson AM. Method for measuring cerebral dysfunction in patients with liver disease. Br Med J 1970;2:633-6.
69. Gitlin N. Subclinical Portal-Systemic Encephalopathy. Am J Gastroenterol 1988;82:8-11.
70. Rikkers L, Jenko P, Rudman D, Freides D. Subclinical hepatic encephalopathy: detection, prevalence, and relationship to nitrogen metabolism. Gastroenterology 1978;75:462-9.
71. Quero JC, Hartmann IJC, Meulstee J, Hop WCJ, Schalm SW. The diagnosis of subclinical hepatic encephalopathy in patients with cirrhosis using neuropsychological tests and automated electroencephalogram analysis. Hepatology 1996;24:556-60.95.
72. Quero JC, Schalm SW. Subclinical hepatic encephalopathy. Sem Liver Disease 1996;16:321-8
73. Weissenborn K, Scholz M, Hinrichs H et al. Neurophysiological assessment of early hepatic encephalopathy. Electroenceph Clin Neurophysiol 1990;75:289-95.
74. Groeneweg M, Quero JC, Essink-Bot ML, Hop WCJ, Schalm SW. The effect of subclinical hepatic encephalopathy on the quality of life. In: Record CO, Al Mardini H editors. Advances in hepatic encephalopathy and metabolism in liver disease. Ipswich, UK: Ipswich Book Company; 1997. P. 311-7.
75. Tarter RE, Hegedus AM, van Thiel DH et al. Nonalcoholic cirrhosis associated with neuropsychological dysfunction in the absence of overt evidence of hepatic encephalopathy. Gastroenterology 1984;1421-7.
76. Gilberstadt SJ, Gilberstadt H, Zieve L, Buegel B, Collier RO, McClain CJ. Psychomotor performance defects in cirrhotic patients without overt encephalopathy. Arch Intern Med 1980;140:519-21.
77. Van der Rijt CCD, Schalm SW, de Groot GH, de Vlieger M. Objective measurement of hepatic encephalopathy by means of automated EEG analysis. Electroencephalograph Clin Neurophysiol 1984;57:423-26.
78. Van Leusen R. Results of protein and non-protein loading tests in patients with hepatocerebral intoxication. Digestion 1972 (suppl);45:244-5.

Part 1

Studies on the pathophysiology

Neuronal and glial marker proteins in hepatic encephalopathy associated with acute liver failure and acute hyperammonemia in the rabbit.

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Abstract

Neuronal and glial cell marker proteins were quantified in order to evaluate the possibility of increased proteolysis in the brain of rabbits with acute liver failure and acute hyperammonemia. Acute liver failure was induced by a two-stage devascularisation procedure. Acute hyperammonemia was induced by a prolonged infusion of ammonium acetate, which simulates the plasma ammonia level in acute liver failure. Control animals received an infusion of sodium/potassium acetate. After development of severe encephalopathy, the animals were sacrificed (13.7 ± 1.3 hours in rabbits with acute liver failure and 20.2 ± 0.8 hours in rabbits with hyperammonemia) (x ± S.E.M.) and brains were dissected into cerebral cortex, hippocampus, cerebellum and brain stem. The total protein content and the concentration of neuronal cell marker proteins NSE (neuron specific enolase), NF- 68 and NF-200 (68 kD and 200 kD neurofilament polypeptides) and the glial cell marker proteins GFAP (glial fibrillary acidic protein) and S-100 were determined. Total protein content was decreased in brain stem in acute hyperammonemia only. The content of neuronal and glial cell markers was not affected in either of the two conditions. However, low molecular weight proteolytic fragments of the NF 68kD polypeptide were observed in the hippocampus of three out of six animals in both experimental groups. No proteolytic degradation was observed for GFAP. The results show that, in experimental encephalopathy associated with acute liver failure and acute hyperammonemia, no major changes occur in the marker proteins. The finding of proteolytic fragments of the NF 68kD polypeptide indicate that the neuronal population is affected prior to glial alterations. These findings are in agreement with the concept that acute hepatic encephalopathy is reversible and induces only slight structural changes.

Introduction

Hepatic encephalopathy is a neuropsychiatric syndrome caused by serious liver disease. Hyperammonemia appears to be an important causative factor. [1] Ammonia disturbs normal brain function in many ways, [1,2] but the precise mechanism of encephalopathy induction is unknown. We have concentrated on the interference of hyperammonemia with excitatory neurotransmission.[3,4] The amino acid glutamate is the most common excitatory neurotransmitter [5] and the metabolism of glutamate is closely related to that of ammonia. [6,7] Increased extracellular concentrations of glutamate were found in the brains of rabbits with acute ischemic liver necrosis, galactosamine-induced liver necrosis and acute hyperammonemia and in the cerebrospinal fluid of rats with acute ischemic liver necrosis. [3,4,8] Furthermore, increased glutamate concentrations are found in the cerebrospinal fluid of patients with hepatic encephalopathy. [9,10,11]

This indicates that the receptors are exposed to increased glutamate levels in hyperammonemia. In vitro experiments suggest that hyperammonemia compromises glutamate re-uptake by astrocytes. [12,13] Moreover, astrocytes are critical for ammonia metabolism [7,13] and the most characteristic neuropathological finding in hepatic encephalopathy is the transformation of astrocytes in grey matter into Alzheimer type II glia. [13-15] The extracellular brain glutamate concentration is found to increase 3.5-10 fold in brain ischemia [16] and this is considered crucial to the development of neuronal deficits and cell death in stroke, hypoglycemia and epilepsy. [17] In ischemia, glutamate toxicity is mainly mediated through activation of postsynaptic receptors. The neurons swell within minutes [18], and die within a day due to excessive calcium influx. [19,20] The extracellular brain glutamate concentration is increased 2-5 fold in experimental acute liver failure. [3,4] Consequently, glutamate neurotoxicity may be of importance also in the pathogenesis of hepatic encephalopathy.

Glutamate receptor mediated neurotoxicity induces degenerative and/or proliferative changes in the brain. The concentration of neuronal and glial cell marker proteins have been used to monitor these effects. These markers include: neuron specific enolase (NSE), a neuronal cytoplasmatic enzyme [21], NF-68 and NF-200, two polypeptide-fragments of neuronal intermediary filaments [22], glial fibrillary acidic protein (GFAP), a fibrillary component of the glial intermediary filament [23] and S-100 which is a glial cytoplasmatic protein. [21]

The appearance of breakdown products of neuronal and glial intermediary filaments is a sensitive indicator of excitatory cell injury. [24] This report concerns degenerative and proliferative changes in neurons and glial cells in experimental liver acute failure and acute hyperammonemia.

Animals and methods

Animals. Nineteen New Zealand white rabbits weighing 2-3 kg were used. Acute liver failure (ALF) was induced by a two-stage liver devascularisation procedure as described earlier. [25,26] Under anesthesia a laparotomy was performed: a loose ligature was placed around the hepatoduodenal ligament and guided through a plastic tube through the abdominal wall to the subcutaneous layer of the left subcostal region, and a small-diameter (5 mm) side-to-side portacaval shunt was constructed. During this procedure the superior mesenteric artery was clamped to reduce splanchnic blood stasis. To correct acidosis after release of the vascular clamps 5 ml 8,4% sodium bicarbonate were given intravenously. Postoperatively the rabbits were given 50 ml 10% glucose subcutaneously, followed by standard laboratory chow and water ad libitum.

The second day after the operation acute liver failure was induced by tightening the loose ligature around the hepatoduodenal ligament after giving 50 mg amoxicillin intravenously. The rabbits were subsequently placed in a restraining box. To prevent hypoglycemia 10% glucose was given intravenously, starting at a volume of 3 ml/hr and adjusted according to the plasma glucose level when necessary. When survival after induction of liver ischemia was less than 6 hours, death was considered to be related to postoperative complications. Only rabbits surviving for at least 6 hours were included for analysis. Acute hyperammonemia (AMM) was induced by a prolonged intravenous ammoniumacetate (NH_4Ac) infusion simulating the plasma ammonia pattern of rabbits with acute liver failure. [26] After insertion of a Venflon cannula (diameter 0.8 mm, Viggo, Helsingborg, Sweden) into an earvein, NH_4Ac was infused at a constant volume of 6 ml/hr, starting with an initial dose of 0.8 mmol/kg/hr, which was subsequently increased by 0.2 mmol/kg/hr every two hours. After 16 hours the dose remained constant. Control rabbits (C) received a sodium-potassium acetate (NaKAc) solution, which was infused as in the NH_4Ac experiments.

Laboratory measurements. Arterial blood samples were taken at 0,1 and 2 hours, and every two hours thereafter for ammonia determination, using the enzymatic method. [27] Blood glucose levels were measured hourly (Haemoglucotest, Boehringer, Germany).

Clinical signs of encephalopathy. At regular time-intervals the rabbits were put into a large cage for clinical evaluation. Rabbits with acute liver failure exhibit two easily recognizable stages of encephalopathy. [28] Stage A is characterized by a disturbed righting reflex: the animal will not get up immediately when placed on its side. In stage B the rabbit lays in the cage and cannot achieve to the sitting position, even after stimulation, and usually cannot lift its head. All rabbits with acute liver failure and all rabbits receiving NH_4Ac were sacrificed during encephalopathy stage B. The rabbits receiving NaKAc were sacrificed at the same time as the rabbits receiving NH_4Ac. All animals were sacrificed through decapitation.

Brain sampling. Brains were rapidly removed and dissected. Dissection was performed on a plastic plate placed on ice. Cerebral cortex (left and right fronto-temporal parts), hippocampus (left and right), cerebellum and brain stem were dissected. Immediately thereafter samples were packed in plastic bags, which were stored at -70°C untill further analysis.

Quantitative analysis of neuronal and glial cell marker proteins. Brain samples were sonified at 90°C and a dot immunobinding procedure was performed:[24,29] brain samples were diluted in buffer (120 mM KCl, 20 mM NaCl, 2 mM $NaHCO_3$ and 5 mM hepes, pH 7.4/0.7% Triton X-100 (vol/vol) to contain ca. 0.5 µg total protein per µL. Using the Minifold II slot-blot apparatus as a template, 20 µL samples were blotted onto nitrocellulose membrane filters. The filters were fixed in 10% acetic acid and 25% 2-propanol. The remaining reactive sites on the filters were blocked with a blocking solution containing 0.5% gelatin. The filters were incubated overnight at room temperature in an antibody solution (blocking solution, 0.1% Triton X-100 and antisera). The antibody preperations were diluted as follows: S-100, 1/500; GFA, 1/2500; NSE, 1/500; NF-68, 1/500 and the supernatant of the monoclonal antibody FE3 against NF-200, 1/100. The specificities of the antibodies have been described in detail. [23] After antibody incubation, the filters were reacted with ^{125} I-protein A in the blocking solution containing 0.1% Triton X-100 at a radioactivity of 150,000-200,000 cpm/ml. Finally the filters were dried, cut and assayed for radioactivity in a gamma counter (Automatic Gamma Counter and Sample Change System 54, Nuclear Data Inc.). For the determination of the NF-200 polypeptide with a monoclonal antibody, rabbit immunoglobulins to mouse immunoglobulins (diluted 1:1000 in the antibody solution) were used as bridging antibodies since protein A binds very weakly to mouse immunoglobulins. The amount of each of the specific proteins in the unknown samples were quantified. First, a reference sample was prepared from a mixture of all the control samples from the frontotemporal cerebral cortex and standard curves were constructed by dilution. The amount of protein was found by interpolation on the standard curves. It was expressed in arbitrary units (AU), defined as the amount of protein in 20 µL of the reference sample. The total protein concentration in the samples was determined with a commercial assay (Pierce) in microtiter plates. Bovine serum albumin was used as standard. [29]

Qualitative analysis of neuronal and glial intermediary filaments. Equal amounts of proteins applied to SDS-PAGE gels were separated by electrophoresis with a linear 6-12 % acrylamide gradient. [30] The proteins were then tranferred to 0.45 µm nitrocellulose membranes (Schleicher & Schuell, Keen, NH, USA) for antibody incubation. [31] The antibody preparations were diluted in the following manner: rabbit anti-human GFA, 1/10000 and rabbit anti-bovine neurofilament NF 68, 1/1000. Preparation of a rabbit antisera used in this study, as well as certification of their specificities, were described elsewhere. [32] The goat-anti-rabbit IgG-alkaline phosophates as the secondary antibody and BCIP/NBT as substrate (BIO-RAD, Richmond, CA, USA).

Necropsy. Necropsy was performed to exclude gross pathological abnormalities. In the animals with acute ischemic liver cell necrosis the liver was examined carefully to confirm tightening of the ligature.

Statistics. The results are presented as mean ± S.E.M. For statistical analysis unpaired Student's *t* test and the Student-Newman-Keuls procedure was used. Statistical significance denotes $p<0.05$.
All experiments were approved by the Ethical Committee on Animal Research of the Erasmus University Rotterdam.

Results

Encephalopathy due to acute liver failure was characterized by complete loss of spontaneous activity, impaired body posture, absence of the righting reflex, decreased muscle tone and a diminished reaction to a painful stimulus. Rabbits with encephalopathy from acute hyperammonemia exhibited similar symptoms, but also had severe ataxia. All animals with acute liver failure and all animals with acute hyperammonemia developed encephalopathy stage B, after 13.7 ± 1.3 and 20.2 ± 0.8 hours respectively (n=6, mean ± S.E.M., $p < 0.05$).
Two rabbits with acute liver failure died just after stage B was recognised. Control studies were performed for 18.8 ± 0.8 hours. During the experiments increases of arterial ammonia levels in the rabbits with acute liver failure and acute hyperammonemia were similar in pattern. At the start of the experiments ammonia levels were significantly higher in ALF-rabbits (120 ± 24 µmol/l), due to their portacaval shunt, compared to AMM-rabbits (49 ± 2.8 µmol/l) or CON-rabbits (51 ± 3.8 µmol/l)($p<0.05$). During encephalopathy stage B the plasma ammonia levels in ALF-rabbits (518 ± 97 µmol/l) and AMM-rabbits (535 ± 117 µmol/l) did not differ significantly ($p>0.05$) (table 1).

Table 1 Mean arterial ammonia during acute liver failure, acute hyperammonemia and control experiments

	T=0	T=4 hours	T=8 hours	T=12 hours	T end
CON	51 ± 3.8	40 ± 4.8	33 ± 0.8	29 ± 1.9	35 ± 3.4
ALF	120 ± 24*	247 ± 26*	313 ± 37*	481 ± 109*	518 ± 97*
AMM	49 ± 2.8	216 ± 23*	315 ± 34*	370 ± 39*	535 ± 117*

Ammonia levels are expressed in µmol/L. CON: controls, ALF: acute liver failure; AMM: ammonia infusion. T=0: baseline; T=4,8 and 12 hours: time after baseline; T end: encephalopathy stage B (end of experiment). *$p < 0.05$ vs. controls (Student Newman Keuls test).

Total protein content was measured of cerebral cortex, hippocampus, brain stem and cerebellum (table 2). During acute liver failure no changes of the total protein content were found. During acute hyperammonemia total protein content of the brain stem was found to be decreased compared to the control rabbits ($p<0.05$); no changes were found in cortex, hippocampus and cerebellum.

Table 2 Brain total protein content after acute liver failure, acute hyperammonemia and control experiments

	CON (n=6)	ALF (n=6)	AMM (n=6)
cortex [1]	73 ± 1.5	73 ± 1.4	73 ± 1.2
hippocampus [1]	83 ± 1.1	86 ± 1.3	81 ± 1.4
cerebellum	72 ± 0.4	74 ± 2.3	70 ± 1.3
brainstem	66 ± 1.0	66 ± 1.1*	60 ± 1.9*

Total protein content is expressed in µg/mg wet weight ± S.E.M. CON: controls; ALF: acute liver failure; AMM: ammonia infusion.[1] mean of sample of right and left side.

The amounts of the neuronal (NSE, NF68 and NF 200) and glial (GFAP and S-100) cell marker proteins of cerebral cortex and hippocampus during acute liver failure and acute hyperammonemia were not different from those found for control rabbits (table 3). Similar results were obtained for the cerebellum and brain stem (not shown).

Sonified brain samples containing equal amounts of the NF 68kD polypeptide and the GFAP from the hippocampus were investigated by the immunoblot technique using polyclonal rabbit antisera against the polypeptides. [24] In both experimental groups, three out of six experimental animals showed a visual increase of low molecular breakdown products of the neurofilament 68 kD polypeptide with molecular weights of 46 and 48 kD compared to the controls. The increase seemed to be more pronounced among the animals infused with ammonia. No alterations of the immunoblot staining pattern of GFAP in hippocampus from these animals were detectable (not shown).

Table 3 Neuronal and glial marker proteins after acute liver failure, acute hyperammonemia and control experiments

	CON (n=6)	ALF (n=6)	AMM (n=6)
cerebral cortex			
GFAP	0.91 ± 0.11	0.80 ± 0.04	0.93 ± 0.16
S-100	0.95 ± 0.04	0.98 ± 0.04	0.96 ± 0.06
NSE	0.79 ± 0.03	0.84 ± 0.03	0.78 ± 0.04
NF 68	0.84 ± 0.04	0.83 ± 0.03	0.94 ± 0.06
NF 200	0.86 ± 0.06	0.85 ± 0.07	0.85 ± 0.08
hippocampus			
GFAP	0.93 ± 0.10	0.82 ± 0.04	0.95 ± 0.11
S-100	0.94 ± 0.05	0.95 ± 0.03	0.93 ± 0.06
NSE	0.84 ± 0.04	0.86 ± 0.05	0.78 ± 0.02
NF 68	0.83 ± 0.02	0.88 ± 0.06	0.81 ± 0.05
NF 200	0.70 ± 0.05	0.69 ± 0.03	0.69 ± 0.06

Proteins are expressed in arbitrary units (AU) ± S.E.M., defined as the amount of protein in 20 µL of a reference sample (see Methods). CON: controls, ALF: acute liver failure; AMM: ammonia infusion.

Discussion

The concentration of neuronal (NSE, NF-68 and NF-200) and glial (GFAP and S100) cell markers was unaffected in experimental encephalopathy due to acute liver failure and acute hyperammonemia; the total protein content was lowered only in the brain stem of animals infused with ammonia. However, an increased amount of low molecular breakdown products of NF 68kD was found in the hippocampus of both treated groups. The degradation pattern of NF68 is similar to that induced by calcium activated neutral proteases in vitro, [33] since the major breakdown products have an approximate molecular weight of 50 kD. Similar breakdown products were observed after in vivo stimulation of hippocampal NMDA receptors in rats. [34]

Changes in glial proteins are the principal finding in hepatic encephalopathy. Cultured astrocytes showed a selective decrease in GFAP after four days of exposure to 10 mM ammonium chloride [35] and there was a selective loss of GFAP in the grey matter of patients who died with liver cirrhosis and Alzheimer type II gliosis. [36,37] Alzheimer type II astrocytes are not seen before 5 - 10 days of treatment. [15,38] The mechanism for this loss of GFAP is unknown. Increased calcium-dependent proteinase activity and decreased protein synthesis have been proposed. [35,39] This specific loss of GFAP is a "marker" for hepatic encephalopathy: other pathological conditions invariably show an increase in GFAP. [13] Predominantly structural proteins appear to be affected in hepatic encephalopathy, since the content of S-100 is not

altered. [40,41] The exposure time (less than 24 hours) in the present study may have been too short for quantitative changes in the proteins to occur. The qualitative change in NF68 seen in the hippocampus of animals with acute liver failure and acute hyperammonemia indicates the involvement of calcium-activated proteolytic enzymes. This could be the result of (early) glutamate neurotoxicity; [18] however, there is no evidence of marked neurofilament degeneration in this study. Whether these findings are of importance for the pathogenesis of hepatic encephalopathy remains uncertain.

More experimental work on this issue is needed in animal models simulating chronic hepatic encephalopathy. However, the results are in agreement with the concept that acute encephalopathy due to acute liver failure and acute hyperammonemia is a reversible condition, which is not characterized by extensive structural changes in the brain.[42]

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References

1. Butterworth RF. Pathophysiology of hepatic encephalopathy; the ammonia hypothesis revisited. In: Bengtsson F, Jeppsson B, Almdal T, and Vilstrup H. (eds.) Progress in hepatic encephalopathy and metabolic nitrogen exchange CRC Press 1991, pp. 9-24.
2. Raabe W. Synaptic transmission in ammonia intoxication. Neurochem Pathol 1987;6:145-66.
3. De Knegt RJ, Schalm SW, van der Rijt CCD, Fekkes D, Dalm E, Hekking I. Extracellular brain glutamate during acute liver failure and during acute hyperammonemia simulating acute hepatic failure: an experimental study based on in vivo brain dialysis. J Hepatol 1994; 20: 119-26.
4. Hamberger A and Nystrom B. Extra- and intracellular amino acids in the hippocampus during development of hepatic encephalopathy. Neurochem Res 1984; 9:1181-92.
5. Fonnum F. Glutamate: a neurotransmitter in mammalian brain. J Neurochem 1984;42:1-11.
6. Cooper, AJL and Plum F. Biochemistry and physiology of brain ammonia. Physiol. Rev 1987;67:440-519.
7. Kaneko T, Shigemoto R, and Mizuno N. Metabolism of glutamate and ammonia in astrocyte: an immunocytochemical study. Brain Res 1988;457:160-64.
8. Swain MS, Bergeron M, Audet R, Blei AT, Butterworth RF. Monitoring of neurotransmitter amino acids by means of an indwelling cisterna magna catheter: a comparison of two rodent models of fulminant liver failure. Hepatology 1992;16:1028-35.
9. Van Sande M, Mardens Y, Adriaenssens K, Lowenthal A. The free amino acids in human cerebrospinal fluid. J Neurochem 1970; 17: 125-35.
10. Watanabe A, Takei N, Higashi T. Glutamic acid and glutamine levels in serum and cerebrospinal fluid in hepatic encephalopathy. Biochem Med 1984;32:225-31.
11. Vergara F, Plum F, Duffy TE. α-Ketoglutarate: increased concentrations in the cerebrospinal fluid of patients with hepatic coma. Science 1974;183:81-3.
12. Mena EE, Cotman CW. Pathologic concentrations of ammonium ions block L-glutamate uptake. Exp

Neurol 1985;89: 259-63.

13. Norenberg MD. The astrocyte in liver disease. In: Federoff S and Hertz L. (eds.) Advances in cellular biology 2. New York Academic Press, 1981, pp. 303-52.
14. Adams RD and Foley JM. The neurological disorder associated with liver disease. In: Merritt HH, Hare CC. (eds.), Proc Ass Res Nerv Ment Dis 1953;32:198-237.
15. Norenberg MD. A light and electron microscopic study of experimental portal-systemic (ammonia) encephalopathy. Progression and reversal of the disorder. Lab Invest 1977;36:618-27.
16. Benveniste H, Drejer J, Schousboe A, and Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia measured by intracerebral microdialysis. J Neurochem 1984;43:1369-74.
17. Rothman SM, Olney JW. Excitotoxicity and the NMDA receptor. TINS 1987;10:299-302.
18. Choi DW. Ionic dependence of glutamate neurotoxicity. J Neurosci 1987;7: 369-79.
19. MacDermott AB, Mayer ML, Westbrook GL, Smith SJ and Barker JL. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. Nature 1986;321:519-22.
20. Schanne FAX, Kane AB, Young EE, Farber JL. Calcium dependence of toxic cell death: a final common pathway. Science 1979;206: 700-2.
21. Moore BW. A soluble protein characteristic of the nervous system. Biochem Biophys Res Comm 1965;19 :739-44.
22. Liem RKH. Simultaneous separation and purification of neurofilament and glial filament proteins from brain. J Neurochem 1982;38:142-50.
23. Eng LF, Veanderhaeghen JJ, Bignamie A, Gerstl B. An acidic protein isolated from fibrous astrocytes. Brain Res 1971: 28: 351-54.
24. Wang S, Lees, GJ, Rosengren LE, Karlsson JE, Stigbrand T, Hamberger A, Haglid KG. The effect of a NMDA lesion in the hippocampus on glial and neuronal marker proteins. Brain Res 1991;541: 334-41.
25. Fick TE, Schalm SW, and De Vlieger M. A surgical model of fulminant hepatic failure in the rabbit: different effects of end-to-side versus small-diameter side-to side portacaval shunt. Eur Surg Res 1987;19:276-82.
26. Fick TE, Schalm SW and De Vlieger M.Continuous intravenous ammonia infusion as a model for the study of hepatic encephalopathy in rabbits. J Surg Res 1989;46:221-225.
27. Da Fonseca-Wolheim F. Direkte plasma ammonia bestimmung ohne enteiweissung. Z Klin.Chem.Klin Biochem 1973;11:426-31.
28. Van der Rijt CCD, De Knegt, RJ, Schalm SW, Terpstra OT, Mechelse K. Flumazenil does not improve hepatic encephalopathy associated with acute ischemic liver failure in the rabbit. Metabol Brain Dis 1990; 5:131-41.
29. Wang S, Rosengren LE, Karlsson JE, Stigbrand T, Haglid KG. A simple quantitative dot-immunobinding assay for glial and neuronal marker proteins in SDS-solubilized brain tissue extracts. J Neurosci Meth 1990;33:219-27.
30. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227: 680-85.
31. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: proceure and some applications. Proc Natl Acad Sci 1979;6: 4350-54.
32. Karlsson J-E, Rosengren LE and Haglid KG. Polyclonal antisera to the individual neurofilament triplet proteins: a characterisation using ELISA and immunoblotting. J Neurochem 1989;53:759-65.
33. Malik MN, Sheikh AM, Febko MD and Wisniewski HM. Purification and degradation of purified neurofilament proteins by the brain calcium-activated neuronal proteases. Life Sciences 1986;39:1335-43.
34. Wang S, Lees JL, Rosengren LE, Karlsson JE, Hamberger A, Haglid KG. Proteolysis of filament proteins

in glial and neuronal cells after in vivo stimulation of hippocampal NMDA receptors. Neurochem Res 1992;17:1005-9.

35. Norenberg MD, Neary JT, Norenberg LOB, McCarthy M. Ammonia induced deecrease in glial fibrillary acidic protein in cultured astrocytes. J Neuropathol Exp Neurol 1990;49:399-405.
36. Kretzschmar HA, DeArmond SJ and Forno LS. Measurement of GFAP in hepatic encephalopathy by ELISA and transblots. J Neuropathol Exp Neurol 1985;4: 459-71.
37. Sobel RA, DeArmond, SJ, Forno LS, Eng LF. Glial fibrillary acidic protein in hepatic encephalopathy. An immunocytochemical study. J Neuropathol Exp Neurol 1981; 40:625-32.
38. Gregorios JB, Mozes LW, Norenberg LOB and Norenberg MD. Morphologic effects of ammonia on primary astrocyte cultures. I. Light microscopic studies. J Neuropath Exp Neurol 1985;44:397-403.
39. DeArmond SJ, Fajardo M, Naughton SA, and Eng LF. Degradation of glial fibrillary acidic protein by a calcium dependent proteinase: an electroblot study. Brain Res 1983; 262: 275-82.
40. Kimura T, and Budka H. Glial fibrillary acidic protein and S-100 protein in human hepatic encephalopathy: immuno-cytochemical demonstration of dissociation of two glial-associated proteins. Acta Neuropathol 1986;70:17-21.
41 Redinbaugh MG, Turley RB. Adaptation of the bicinchoninic acid protein assay for use with microtiter plates and sucrose gradient fractions. Ann Biochem 1986;53:267-71.
42. Sherlock S. Diseases of the liver and biliary system. (8th ed.), Blackwell Scientific Publications, Oxford, England, 1989, pp. 95-115.

The NMDA-receptor antagonist MK-801 does not improve survival of rabbits with encephalopathy associated with acute liver failure and acute hyperammonemia.

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Abstract

Extracellular brain glutamate levels are increased in experimental models of acute hyperammonemia and acute liver failure, and glutamate over-exposure may be associated with these syndromes. Experiments were performed to study the effect of the N-methyl-D-aspartate glutamate receptor antagonist MK-801 (dizocilpine) on the development of encephalopathy and survival of rabbits with acute liver failure and acute hyperammonemia.

MK-801 (0.05 mg/kg/hr) or placebo was randomly allocated to 36 rabbits (18 MK-801; 18 placebo) infused with ammonium acetate to induce hyperammonemia simulating that of acute liver failure, to 18 rabbits (11 MK-801; 7 placebo) that had undergone a liver devascularisation procedure to induce acute liver failure, and to nine control rabbits (4 MK-801;5 placebo) infused with sodium-potassium acetate. The development of encephalopathy was significantly enhanced by MK-801 (ammonia infusion group: 13.7±6.6 vs. 20±4.3 hours, acute liver failure group: 4.2±3.9 vs. 11±2.6 hours); control rabbits treated with MK-801 also showed signs of encephalopathy. Survival was not affected by MK-801 treatment (ammonium infusion group: 22.7 ± 5.3 vs. 20.9 ± 4.3 hours; acute liver failure group: 14 ± 7.8 vs. 12 ± 2.3 hours, and control group: 29.5 ± 1 vs. 28.8 ± 1.5 hours (MK-801 vs. placebo)).

MK-801 in maximum tolerable dosis did not prevent development of encephalopathy nor improved survival of rabbits with acute hyperammonemia and acute liver failure.

Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome in patients with liver failure, characterized by disturbance of mental state, neuromuscular signs and electroencephalographic abnormalities. [1] Disturbances in ammonia metabolism are thought to be strongly associated with the pathogenesis of HE, although the precise mechanism of ammonia toxicity is unknown. Experimental models of acute hyperammonemia or acute liver failure have indicated that extracellular brain glutamate levels are increased, [2-4] probably as a result of an ammonia-induced decrease in glutamate re-uptake into astrocyte. [4,5] Increased post-synaptic stimulation by glutamate down-regulates non-NMDA-glutaminergic receptors (kainate and AMPA) in experimental acute liver failure, [6] which results in a relative over-exposure of the NMDA receptor. [5] Over-exposure of the NMDA receptor may lead to increased calcium influx intracellularly, disturbance of ion homeostasis, and cellular swelling. Eventually this neurotoxicity leads to cell death. [7] We hypothesized that glutamate neurotoxicity may have a role in the pathogenesis of hepatic encephalopathy. To test this hypothesis, a placebo-controlled experiment was performed to study the effect of blocking NMDA-receptors of rabbits with encephalopathy as a result of acute liver failure and acute hyperammonemia, by treatment with the NMDA-receptor antagonist MK-801.

Animals and methods

Animals. New Zealand white rabbits weighing 2-3 kg were used. Acute hyperammonemia (AMM) was induced by prolonged intravenous ammonium acetate (NH_4Ac) infusion. After insertion of a Venflon cannula (diameter 0.8 mm, Viggo, Sweden) into an earvein, NH_4 Ac was infused at a constant volume of 6 ml/hr, starting with an initial dose of 0.8 mmol/kg/hr, which was subsequently increased by 0.2 mmol/kg/hr every two hours. After 16 hours the dose remained constant. [8,9]

Acute liver failure (ALF) was induced by a two-stage liver devascularization procedure. [9,10] Under anesthesia a laparotomy was performed: a ligature was placed around the hepatoduodenal ligament and guided through a plastic tube through the abdominal wall to the subcutaneous layer of the left subcostal region, and a small-diameter (5 mm) side-to-side portacaval shunt was constructed. The superior mesenteric artery was clamped to reduce splanchnic blood stasis. To correct acidosis after release of the vascular clamps 5 ml 8.4% sodium bicarbonate was given intravenously. Postoperatively the rabbits were fed with standard laboratory chow and water ad libitum. The second day after the operation, acute liver failure was induced by tightening the ligature around the hepatoduodenal ligament, after giving 50 mg amoxicilline intravenously. The rabbits were subsequently placed in a restraining box. To prevent hypoglycemia 10% glucose was given intravenously, starting at a volume of 3ml/hr and which was adjusted according to the plasma glucose level when necessary.

Control rabbits (CON) received a sodium-potassium acetate (NaKAc) solution, which was infused, as far as the acetate concentration was concerned, as in the rabbits infused with NH_4Ac. Blood gasses were measured every 4 hours and blood glucose levels every two hours (Haemoglucotest, Boehringer, Germany). Metabolic acidosis was corrected at a blood pH levels of 7.0, by giving one bolus of 5 ml 8.4% sodium bicarbonate intravenously.

Study design. A placebo-controlled study was designed; in each experimental group animals were treated with MK-801 or placebo. Two rabbits of one experimental group were studied simultaneously. MK-801 or placebo treatment was allocated to each animal by randomization, performed by a laboratory analyst who also prepared the syringes containing the MK-801 or placebo solution. The study investigators were not aware of which treatment was given. The outcome measures were: time needed to develop encephalopathy and survival time. Necropsy was performed in all animals to exclude gross morphological abnormalities of the lungs and the intestine, and to confirm the tightening of the ligature in the ALF rabbits. Immediately after sacrifice or spontaneous death, blood was drawn for bacterial cultures. All experiments were approved by the ethical committee on animal experiments of the Erasmus University Rotterdam.

Study medication. MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclo-hepten-5,10-imine maleate] (dizocilpine) was provided by Merck Sharp & Dohme Research Laboratories, New Jersey, USA. Results of a dose-finding study with intravenous doses of MK-801(ranging from 0.01 to 0.1 mg/kg/hr) showed increasing side effects from a dose of 0.05 mg/kg/hr (data not shown). Thus, MK-801 was dissolved in 0.9% saline to yield a dosage of 0.05 mg/kg/hr. MK-801 was administered intravenously at a constant volume of 4 mL/hr. Placebo treated animals received 4 ml of 0.9% saline/hour. The study medication was administered simultaneously with the infusion of NH_4Ac (AMM group), NaKAc (CON group) or with the infusion of glucose (ALF group).

Evaluations.

Clinical Assessment. During the experiments rabbits were kept in a restraining box and at regular time-intervals they were put into a large cage for clinical evaluation. Encephalopathy was graded every two hours: stage A: ataxia or disturbance of the righting reflex; the animal is not getting up immediately when placed on its side; stage B: absence of righting reflex; the animal lays in the cage and cannot come to sitting position, not even after stimulation, and does not lift its head. [11] Rectal body temperature (Philips Digital Thermometer, the Netherlands) was measured at the start of the experiments and at encephalopathy stage B. Respiratory frequency was documented every two hours by observing the animals. Heart rate was measured every two hours by stethoscope.

Biochemical assessment. After insertion of a Venflon cannula into an ear artery, arterial blood samples were taken every two hours for ammonia determination, using the enzymatic method according to Da Fonseca. [12]

Spectral analysis of the electroencephalogram (EEG). Three silver electrodes were implanted directly onto the dura for EEG monitoring. Two electrodes were placed onto the right hemisphere, one anterior (3 mm) and one posterior (11 mm) to the sutura coronaria. The third electrode, the ground electrode, was placed onto the left hemisphere. The electrodes were fixed onto the skull with dental cement. Automated EEG analysis (spectral analysis) was performed as follows [13]: during 100 seconds, variations in the electric potentials between 0.53 and 70 cycles per second in the frontal-occipital lead were registered by an EEG-apparatus (Siemens Elema EEG 10, Sweden). The data entered into a personal computer (Olivetti M24, Italy). To avoid interference of high frequency energy, signals were filtered at 25.6 Hz. The power spectrum was constructed, using Fast Fourier Transformation, and the mean dominant frequency (MDF) was calculated.

Statistical analysis. After closure of the database, the randomization code was broken and intention-to-treat analysis was performed. In addition, per protocol analysis was performed after exclusion of the following rabbits: CON rabbits that died spontaneously during the experiment, AMM rabbits that did not survive for at least 16 hours, and ALF rabbits that did not survive for at least 6 hours. In such cases death was considered not to be due to encephalopathy, but to postoperative complications or to intercurrent disease. [9] Time needed to develop encephalopathy stage A and B and survival time were tested with the Wilcoxon rank sum test. Statistical significance denotes $p < 0.05$.

Results

Survival analysis. A total of 63 animals were enrolled in the study. Nine rabbits in the control group (CON), 18 rabbits in the acute liver failure group (ALF) and 36 rabbits in the ammonia infusion group (AMM) (table 1).

Table 1 Survival of rabbits with acute hyperammonemia and acute liver failure treated with MK-801 or placebo.

group	animals itt (n)	survival (itt) mean ± SD (range)		p-value*	animals pp (n)	survival (pp) mean ± SD (range)		p-value*
CON								
MK-801	4	29.5 ± 1	(28-30)		4	29.5 ± 1	(28-30)	
placebo	5	27.6 ± 2.9	(23-30)	0.27	4	28.8 ± 1.5	(27-30)	0.27
AMM								
MK-801	18	21.8 ± 6.3	(7-31)		17	22.7 ± 5.3	(12-31)	
placebo	18	20.9 ± 4.3	(14-29)	0.47	18	20.9 ± 4.3	(14-29)	0.29
ALF								
MK-801	11	9.6 ± 8.9	(0.5-30)		7	14 ± 7.8	(5-28)	
placebo	7	8.7 ± 5.9	(0.2-15)	0.82	5	12 ± 2.3	(9-15)	0.63

CON: control animals; AMM: ammonia infusion; ALF: acute liver failure; itt: intention to treat; pp: per protocol analysis. * p-value: Wilcoxon rank sum test, MK-801 vs. placebo.

Intention-to-treat analysis showed no difference in survival in either of the three groups, comparing MK-801 treatment with placebo (table 1). For the per protocol analysis, eight rabbits were excluded: in the CON group one rabbit died spontaneously after 23 hours. Post-mortem examination of the lungs showed signs of pneumonia. In the AMM group one rabbit died 7 hours after starting the infusion. Post-mortem examination also showed signs of pneumonia. In the ALF

group 6 rabbits died within two hours after tightening of the ligature. Post-mortem examination showed signs of intestinal ischemia in one rabbit, severe abdominal bleeding in two, and an occluded shunt in one rabbit. Two rabbits in the ALF group died of unknown cause (one was randomized to receive MK-801; the other one placebo). In the per protocol analysis, survival of animals with acute liver failure was markedly longer than in the intention-to-treat analysis, but again in neither of the three experimental groups a difference in survival between MK-801 treated animals and placebo was observed. (table 1, figure 1).

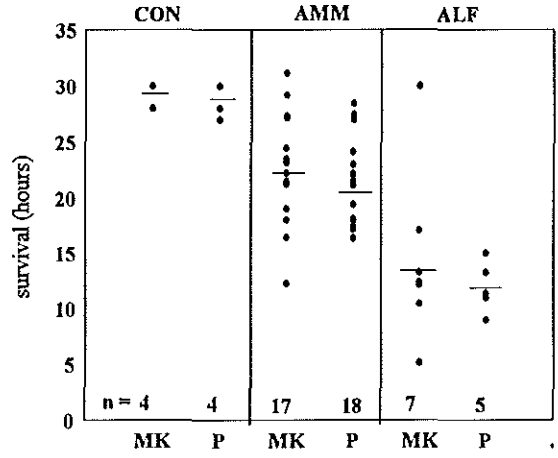


Figure 1 Survival after MK-801 or placebo of the three experimental groups. Survival time after MK-801 treatment of rabbits with encephalopathy as a result of hyperammonemia and acute liver failure (per protocol analysis). Every dot on the graph represents the survival time (hours) of one rabbit. X-axis: MK: MK-801 treatment; P: placebo treatment; CON: control animals; AMM: animals infused with ammonia; ALF: animals with acute liver failure. Horizontal lines indicate the median survival time.

Clinical and biochemical evaluations.

Vital signs. No difference in heart rate, respiratory frequency nor body temperature was found between MK-801 and placebo treated animals (data not shown).

Development of encephalopathy. The results of animals included in the per protocol analysis are listed in table 2. CON animals treated with placebo did not show signs of encephalopathy. However, CON animals treated with MK-801 developed encephalopathy stage A after 2.5±1 hours and stage B after 22.5±8.5 hours. This effect of MK-801 explains that also in the AMM group the time needed to develop encephalopathy stage A and B, and in the ALF group the development of encephalopathy stage B was significantly less in animals treated with MK-801, compared with placebo.

Table 2 Development of encephalopathy stage A and B of rabbits with acute hyperammonemia and acute liver failure treated with MK-801 or placebo (per protocol analysis)

group	number of animals	stage A	p-value*	stage B	p-value*
CON					
MK-801	4	2.5 ± 1		22.5 ± 8.5	
placebo	4	n.d.		n.d.	
AMM					
MK-801	14	2.2 ± 1		13.7 ± 6.6	
placebo	18	17.3 ± 4	<0.001	20 ± 4.3	0.02
ALF					
MK-801	7	1.3 ± 0.5		4.2 ± 3.9	
placebo	5	5.5 ± 3	0.1	11 ± 2.6	0.05

CON: control animals; ALF: acute liver failure; AMM: ammonia infusion. Development of encephalopathy expressed in hours (mean ± SD). * p-value: Wilcoxon rank sum test, comparing MK-801 vs. placebo. n.d.: not detected

Arterial ammonia. The results of animals included in the per protocol analysis are listed in table 3 and in figure 2. The arterial ammonia concentration increased significantly in both AMM group and ALF group, which is in accordance with previous studies, using the same models. [2,8-11] Baseline arterial ammonia levels of ALF animals were higher, as a result of the portacaval shunt, constructed 24 hours before the start of the experiment (96±48 vs. 43±14 μmol/L (mean±SD:$p<0.001$)).

Table 3 Arterial ammonia concentrations (µmol/L) at baseline, at encephalopathy stage A and B, and shortly before death of rabbits with acute hyperammonemia and acute liver failure treated with MK-801 or placebo (per protocol analysis).

group	T0	p-value*	stage A	p-value	stage B	p-value*	Te	p-value
CON								
MK-801	34±7.8		28±3.4		24±1.7		24±3	
placebo	35±5.7	0.91	n.d.		n.d.		26±5	0.67
AMM								
MK-801	42±14		170±55		477±168		715±101	
placebo	43±14	0.81	626±100	<0.01	706±99	0.001	677±146	0.39
ALF								
MK-801	80±23		182±178		276±98		321±156	
placebo	115±64	0.19	241±109	0.59	444±147	0.08	525±188	0.1

CON: control animals; AMM: ammonia infusion, ALF: acute liver failure. Ammonia levels are expressed as mean±SD. T0: baseline values; stage A and B: value at encephalopathy stage A and B; Te: values before death. *p-value: unpaired Student-t test, comparing MK-801 vs. placebo. n.d.: not detected.

At encephalopathy stage A both AMM group and the ALF group had similar ammonia levels (363±247 vs. 212±140 µmol/l (p=0.1)); at encephalopathy stage B ammonia levels were significantly higher in the AMM group, compared with the ALF group (557±183 for AMM vs. 332±136 µmol/l (p=0.002)). The same difference was found shortly before death of the animals (figure 2). The time needed tot develop encephalopathy in AMM rabbits is considerably longer, compared with ALF rabbits. Other factors induced by acute liver failure apart from hyperammonemia enhance the development of encephalopathy and death. [9-11]

CON animals treated with MK-801 had normal arterial ammonia levels at the time of development of encephalopathy. AMM rabbits treated with MK-801 developed encephalopathy stage A and B at significantly lower arterial ammonia levels, compared with placebo treatment. In the ALF group no difference in ammonia levels between MK-801 or placebo treated animals was found (table 3).

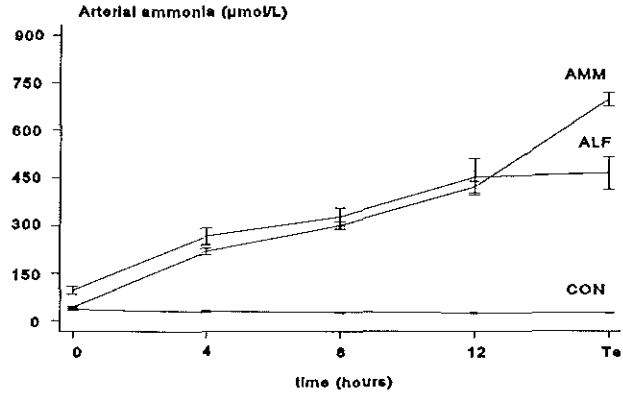
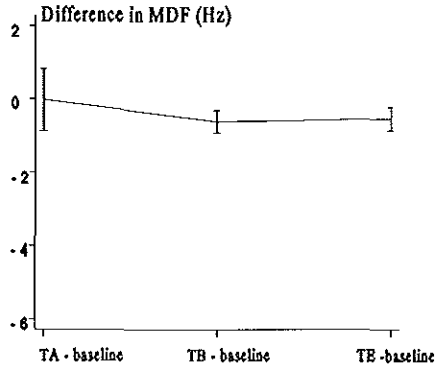


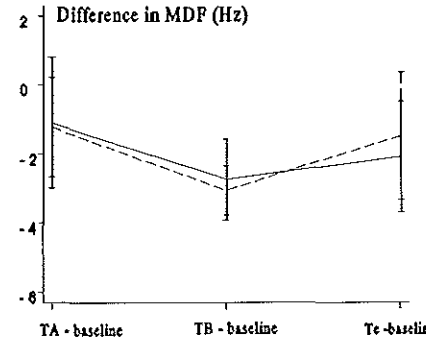
Figure 2 Arterial ammonia levels (µmol/L) of the three experimental groups: MK-801 treated animals and placebo treated animals taken together. Line shows mean arterial ammonia levels (µmol/L)(Y-axis); vertical bars indicate mean ± S.E.M. X-axis: 0: baseline values; 4, 8, 12: values after four, eight and twelve hours, respectively. Te: values shortly before death. CON: control animals; AMM: animals infused with ammonia; ALF: animals with acute liver failure.

Spectral analysis of the EEG.

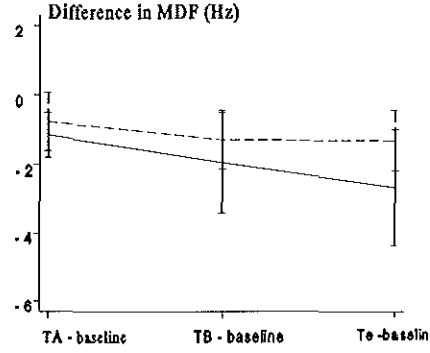
The mean dominant frequency (MDF) of animals included in the per protocol analysis are presented in figure 3. The MDF of CON animals was unaltered during the experiments (figure 3a). The MDF of AMM animals and of ALF animals was significantly decreased at encephalopathy stage B, compared with baseline (for AMM: 3.4±0.7 vs. 5.0±1.1 Hz: $p<0.001$; for ALF: 3.0±0.4 vs. 5.6±1.3 Hz: $p<0.001$), which is in accordance with previous studies, using the same models. [9,11] The MDF of CON animals treated with MK-801 was unaltered during development of encephalopathy stage B, compared with baseline (3.3±0.7 vs. 3.8±0.4 Hz (mean±SD: $p=0.45$). MDF of AMM animals treated with MK-801 was significantly decreased shortly before death, compared with placebo (2.6±0.4 vs. 3.3±1.1 Hz: $p=0.04$) (figure 3b). No significant difference in MDF was found in ALF animals treated with MK-801, compared with placebo (figure 3c).



3a. CON



3b. AMM



3c. ALF

Figure 3: Difference in mean dominant frequency (Hz) of the electroencephalogram of the three experimental groups. Baseline vs. stage A and B and shortly before death. MK-801 compared with placebo. Figure 3a: control animals (CON); figure 3b: animals infused with ammonia (AMM); figure 3c: animals with acute liver failure (ALF). Lines show mean difference in MDF (Hz)(Y-axis), vertical bars indicate mean ± S.D. X-axis: TA-baseline: difference between MDF at encephalopathy stage A and baseline; TB-baseline: difference between MDF at encephalopathy stage B and baseline; Te-baseline: difference between MDF shortly before death and baseline. Dotted line: placebo treatment; solid line: MK-801 treatment. CON animals treated with placebo did not develop encephalopathy (only solid line given).

Discussion

Within the conditions of our study, MK-801, an antagonist of the NMDA receptor, does not improve survival of rabbits with encephalopathy as a result of acute liver failure or acute hyperammonemia. Development of encephalopathy was significantly enhanced in MK-801 treated rabbits and it developed at lower levels of ammonia, compared with placebo treated rabbits. Control rabbits also showed signs of encephalopathy after MK-801 infusion.

Neurologic side-effects of MK-801 have shown to be dose-dependent. [14-16] Kochhar et al.[14] observed slight sedation after an intravenous bolus-injection of 0.2 mg/kg in rabbits, and significant side-effects were seen at 0.4 mg/kg: ataxia, loss of righting reflex and sedation were evident in 5 minutes after injection. Others observed ataxia, circling and lateral head nodding of rats and rabbits after administration of 0.5 mg/kg MK-801 intraperitoneally. [15,16] Marcaida et al. [17] could prevent death of mice and rats with acute hyperammonia through pre-treatment with MK-801. In this animal model of acute ammonia toxicity, intraperitoneal administration of ammonia results in very high ammonia levels within minutes after injection. This rapid increase induces convulsions and death of these animals. The observed effect of MK-801 could be the result of its anti-convulsive activity. [15,16] In our model ammonia increases more slowly - simulating acute liver failure - and convulsions are rarely observed in our model. [9,11]

Our results do not give support to the hypothesis that high extracellular brain glutamate play a role in the pathogenesis of encephalopathy associated with acute liver failure or acute hyperammonemia. However, our results contrast with observations by others: Vogels et al. [18] found improvement in encephalopathy in rats with acute liver ischemia and ammonia infusion by treatment with memantine, also an antagonist of the NMDA receptor. The dose of memantine used in that study was high enough to induce the kind of side-effects we found, and which were found by others, using the same animal. [16] However, no side-effects were reported in this study. This observed difference in tolerability between MK-801 and memantine has also been described in other brain disorders. [19]

So, we have to address the reliability of our findings; firstly, the animal models for acute hyperammonemia and acute liver failure we used are highly reproducible with respect to arterial ammonia levels and to the development of encephalopathy. [9] In these models encephalopathy is accompanied by increased extracellular brain glutamate levels. [3] Secondly, the dose of MK-801 we used was supposed to be high enough to protect neurons from glutamate neurotoxicity. [14,16] The side-effects observed in all groups during MK-801 treatment indicate that we used an effective dose. Thirdly, the placebo-controlled design, the objective elements in the outcome measures and the number of animals in each group greatly limits the chance of selection bias or a type II error responsible for our negative findings.

If glutamate neurotoxicity plays a causal role in the pathogenesis of encephalopathy, blocking of the NMDA receptors should reverse encephalopathy and increase survival. Our results do not give support to the hypothesis that glutamate neurotoxicity has a major role in the pathogenesis of encephalopathy associated with acute hyperammonemia and acute liver failure.
Encephalopathy sets in more rapidly during MK-801 treatment in both experimental conditions, and especially in AMM rabbits, which was objectified by electroencephalography. This observation suggests that decreased glutamatergic neurotransmission rather than glutamate over-exposure is related to the pathogenesis of hepatic encephalopathy. [20,21]

Acknowledgements

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References

1. Conn HO. The hepatic encephalopathies. In: Conn HO, Bircher J, eds. Hepatic encephalopathy: syndromes and therapies. Bloomington. Medi-Ed press, 1994:1-12.
2. De Knegt RJ, Schalm SW, van der Rijt CCD, Fekkes D, Dalm E, Hekking I. Extracellular brain glutamate during acute liver failure and during acute hyperammonemia simulating acute hepatic failure: an experimental study based on in vivo brain dialysis. J Hepatol 1994; 20: 119-26.
3. Bosman DK, Deutz NEP, De Graaf AA. Changes in brain metabolism during hyperammonemia and acute liver failure: results of a comparative H-NMR spectroscopy and biochemical investigation. Hepatology 1990; 12:281-90.
4. Butterworth RF, Le O, Lavoie J, Szerb JC. Effect of portacaval anastomosis on electrically stimulated release of glutamate from rat hippocampal slices. J Neurochem 1991;56:1481-84.
5. Butterworth RF. Hepatic encephalopathy and brain edema in acute hepatic failure: does glutamate play a role? Hepatology 1997;25:1032-34.
6. Michalak M, Butterworth RF. Selective loss of binding sites for the glutamate receptor ligand [^{3}H]kainate and (S)-[^{3}H]5-fluorowillardiine in the brains of rats with acute liver failure. Hepatology 1997;25:631-35.
7. Choi DW. Ionic dependence of glutamate neurotoxicity. J Neurosci 1987;7(2):369-79.
8. Fick TE, Schalm SW, de Vlieger M. Continuous intravenous ammonia infusion as a model for the study of hepatic encephalopathy in rabbits. J Surg Res 1989;221-25.
9. De Knegt RJ, Groeneweg M, Schalm SW, Hekking-Weijma I. Encephalopathy from acute liver failure and from acute hyperammonemia in the rabbit. A clinical and biochemical study. Liver 1994;14:25-31.
10. Fick TE, Schalm SW, de Vlieger M. A surgical model of fulminant hepatic failure in the rabbit: different effects of end-to-side versus small-diameter side-to-side portacaval shunt. Eur Surg Res 1987;19:276-82.
11. Van der Rijt CCD, de Knegt RJ, Schalm SW, Terpstra OT, Mechelse K. Flumazenil does not improve hepatic encephalopathy associated with acute ischemic liver failure in the rabbit. Metab Brain Dis 1990;5:131-41.
12. Da Fonseca-Wohlheim D. Direkte plasma ammonia bestimmung ohne enteiweissung. Z Klin Chem Biochem

1973;11:426-31.

13. Van der Rijt CCD, Schalm SW, De Vlieger M. Objective measurements of hepatic encephalopathy by means of automated EEG analysis. Electroenceph Clin Neurophysiol 1984;57:423-26.
14. Kochhar A, Zivin A, Lyden PD, Mazzarella V. Glutamate antagonist therapy reduces neurologic deficits produced by focal central nervous system ischemia. Arch Neurol 1988;45:148-53.
15. Robinson GB, Reed GD. Effect of MK-801 on the induction and subsequent decay of long-term potentiation in the unanesthetized rabbit hippocampal dentate gyrus. Brain Res 1992;569:78-85.
16. Vezzani A, Serafini R, Stasi MA. Kinetics of MK-801 and its effect on quinolinic acid-induced seizures and neurotoxicity in rats. J Pharmacol Exp Ther 1989;249:278-283.
17. Marcaida G, Felipo V, Hermenegildo C. Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors. Fed. Eur. Biochem. Soc. vol. 296(1), 67-68.
18. Vogels BAPM, Maas MAW. Memantine, a noncompetitive NMDA receptor antagonist improves hyperammonemia-induced encephalopathy and acute hepatic encephalopathy in rats. Hepatology 1997;25:820-27.
19. Kornhuber J, Weller M. Psychogenicity and N-methyl-D-aspartate receptor antagonism: implications for neuroprotective pharmacotherapy. Biol Psychiatry 1997;41:135-44.
20. Groeneweg M, de Knegt RJ, Schalm SW. Neuronal and glial marker proteins in encephalopathy associated with acute liver failure and acute hyperammonemia in the rabbit. Metabolic Brain Disease 1993; 8(2):95-106.
21. Bustos G, Abarca J, Forray MI, Gysling K, Bradberry CW, Roth RH. Regulation of excitatory amino acid release by N-methyl-D-aspartate receptors in rat striatum: in vivo microdialyis studies. Brain Res 1992;585:105-15.

Thyroid hormone metabolism in dogs with portal-systemic encephalopathy as a result of a portacaval shunt and partial hepatectomy.

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Submitted for publication

Abstract

Non-thyroidal illness (NTI) is a common finding in patients with chronic liver disease. Reports have indicated, that NTI may have a role in the pathogenesis of hepatic encephalopathy. To test this hypothesis, thyroid hormones were measured in serum and in cerebrospinal fluid (CSF) of dogs with portal-systemic encephalopathy as a result of a portocaval shunt and partial hepatectomy. In 9 beagle dogs (5 ♂, 4 ♀, median age 1.5 years, mean weight 11.5 kg) a portocaval shunt was constructed (PCS) and a 40 % hepatectomy was performed. 9 pair-fed SHAM operated dogs served as controls. At baseline and at four weeks after surgery the severity of hepatic encephalopathy was graded, and blood samples were drawn for analysis of ammonia, albumin, bile acids, thyroxine (T_4), triiodothyronine (T_3), free T_4 (FT_4) , reverse T_3, T_4-sulphate (T_4S), and T_3-sulphate (T_3S).

PCS dogs developed clinical signs of encephalopathy, hyperammonemia and hypalbuminemia. T_4 and T_3 levels decreased significantly, and T_4S degradation was significantly diminished.

Free T_4 , reverse-T_3 and T_3S levels remained unaltered. In SHAM dogs $_4T$ and $_3T$ were also decreased, which was significant for T_4 only.

Low T_3 and T_4 levels reflect altered plasma protein binding, diminished hepatic T_4 uptake and decreased type 1 iodothyronine deiodinase activity (D1). The changes in T_3 and T_4 levels observed in SHAM dogs is probably the result of caloric deprivation.

The contribution of this altered thyroid hormone metabolism to the pathogenesis of portal-systemic enephalopathy remains speculative. However, T_4 uptake into the brain may be compromised by the same substances that interfere with plasma thyroid hormone binding and with T_4 uptake into the liver. Future research is needed to prove whether altered brain uptake of thyroid hormone into the brain is related to hepatic encephalopathy.

Introduction

Altered thyroid hormone metabolism is a common finding in patients with chronic liver disease. [1,2] Low triiodothyronine (T_3) and low thyroxine (T_4) levels have been described, without clinical signs of hypothyroidism. In general, central regulation of the thyroid gland is preserved in patients with chronic liver disease. The observed changes in thyroid hormone metabolism have therefore been classified as non-thyroidal illness (NTI). [3,4] Studies in patients with chronic liver disease have indicated, that T_4 and T_3 are sensitive markers for the severity of liver disease. [5,6] Moreover, a case-control study performed at our department indicated, that serum total T_3 has strong independent predictive power for hepatic encephalopathy [7]. A recent case-report suggested, that supplementation of thyroid hormone may improve portal-systemic encephalopathy. [8] We therefore hypothesize, that NTI has a role in the pathogenesis of hepatic encephalopathy. To test this hypothesis, we measured thyroid hormones in serum and in

cerebrospinal fluid (CSF) in dogs with portal-systemic encephalopathy as a result of a portocaval shunt and partial hepatectomy.

Animals, materials and methods

Animals and procedures. In 9 beagles (5 ♂, 4 ♀, median age 1.5 years, mean weight 11.5 kg) an end-to-side portocaval shunt was created and a 40% hepatectomy was performed as described before. [9] Each of these dogs (referred to as PCS dogs) was assigned to a control dog of the same gender, and approximately the same weight and age. In the control dogs a sham operation was performed, consisting of clamping of the portal vein for 10 minutes and manipulation of the liver lobes and the structures in the hilar region of the liver (dogs referred to as SHAM). All dogs were housed under natural lighting conditions, had free access to water, and received a moderately protein-restricted canned dog food (14.7% protein dry matter base; K/D ®, Hill's Pet Nutrition, Topeka, Kansas, USA). The PCS dogs were fed ad libitum twice daily. SHAM dogs were pair-fed with the amount of food their partner had eaten the day before. Body weight was recorded weekly. Clinical grading of hepatic encephalopathy was performed once daily by one observer on an ordinal scale: 0: normal; 1: reduced motility and/or mild apathy; 2: severe apathy and/or mild ataxia; 3: salivation, severe ataxia, head pressing, apparent blindness, and/or circling; 4: seizures and/or nearly complete to complete unresponsiveness (coma). [10] Surgical procedures were performed under sterile conditions and under general anaesthesia consisting of a premedication with 0.3 mL/kg of a mixture of droperidol (2.5 mg/mL) and fentanil (0.05 mg/mL) (Thalamonal®, Janssen Pharmaceutica, Tilburg, the Netherlands), induction with sufentanil (Sufenta®, Janssen Pharmaceutica, Tilburg, the Netherlands) 2-2.5 µg/kg IV dosed to effect, and maintenance by artificial ventilation with a mixture of isoflurane N_2O, and O_2 and sufentanil 1-1.5 µg/kg IV. The protocol of this study was approved by the Ethical and Animal Welfare Committee of the Faculty of Veterinary Medicine of the Utrecht University, and dogs were given humane care.

Sampling of blood and cerebrospinal fluid (CSF). Arterial and venous blood samples and CSF were collected after a 12 hour fast before and one month after the surgical procedure. The arterial samples were placed immediately in sodium-EDTA coated tubes on melting ice. Samples were centrifuged at 0-2° C and the plasma ammonia concentration was measured by an enzymatic method (Boehringer Mannheim, Almere, the Netherlands). Venous samples were used for determination of plasma albumin and total bile acids with standard laboratory techniques. The remainder of these samples was frozen at -70°C until analysis of the thyroid hormones. CSF was taken via puncture of the cisterna magna under anaesthesia with propofol (Rapinovet®, Mallinckrodt Veterinary, Aalst, the Netherlands) 1-3 mg/kg IV to effect, and ammonia was measured immediately as described above.

Determination of serum thyroid hormones. Plasma total T_4, T_3 and rT_3 were determined by specific radioimmunoassays, as previously described [11]. Plasma free T_4 was assayed, using Amerlite MAB FT_4 kit (Amersham, Buckinghamshire, UK). Plasma T_4S and T_3S were determined by specific radioimmunoassays essentially as described by Wu [12] and Chopra. [13]

Statistical analysis. Differences in clinical and biochemical parameters were tested with the two-sided Wilcoxon matched pairs signed rank sum test (baseline versus four weeks after surgery), and Wilcoxon rank sum test (PCS versus SHAM: baseline values and values four weeks after surgery). Skewness of data on thyroid hormones, metabolites, and ratios was evaluated with the variance ratio test (F test). Differences of samples with equal variances were tested with the two sample t test (paired data: baseline versus four weeks after surgery; unpaired data: PCS versus SHAM: baseline values and values four weeks after surgery). Differences of skewed data were tested with the two-sided Wilcoxon matched pairs signed rank sum test (baseline versus four weeks after surgery), and Wilcoxon rank sum test (PCS versus SHAM: baseline values and values 4 weeks after surgery). Level of significance denotes $p \leq 0.05$.

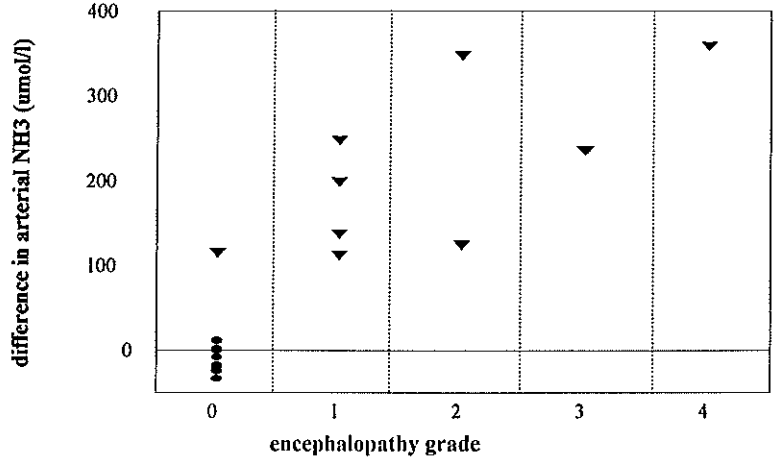

Figure 1: Arterial ammonia concentration versus grade of encephalopathy. X-axis: grade of encephalopathy (ranging from 0 to 4); Y-axis: difference in arterial ammonia level between levels at 4 weeks after PCS construction and baseline values. Levels are expressed in µmol/L. Triangles (▲): PCS dogs; dots (●): SHAM.

Results

Clinical and biochemical parameters are listed in table 1. PCS dogs had severe portal-systemic shunting, as reflected by a significant increase in bile acids and arterial ammonia. Arterial ammonia was significantly increased in PCS dogs at four weeks after surgery, compared with SHAM dogs (239 ± 111 vs. 33 ± 12 µmol/L (p=0.002)). The difference in baseline ammonia levels and ammonia levels at 4 weeks after sugery against the grade of encephalopathy is presented in Figure 1. CSF ammonia levels were signficantly increased in PCS dogs at four weeks after surgery, compared with SHAM dogs. Albumin levels were significantly decreased in PCS dogs at four weeks after surgery (32 ± 2 vs. 26 ± 3.7 g/L: p=0.008). Albumin levels of pair-fed SHAM dogs remained unaltered (table 1).

Body weight decreased significantly in both experimental groups. PCS dogs had clinical signs of encephalopathy four weeks after surgery, ranging from grade 0-4. One PCS dog did not have clinical signs of hepatic encephalopathy at the day of sampling. However, several episodes of encephalopathy were observed in this dog during the post-operative period, and its biochemical profile was similar to the other PCS dogs (arterial ammonia increased from 37 to 151 µmol/L, CSF ammonia increased from 9 to 78 µmol/L, and bile acids increased from 8 to 480 µmol/L). Total thyroxine (T_4), triiodothyronine (T_3), free thyroxine (FT_4), reverse triiodothyronine (rT_3), T_4-sulphate (T_4S) and T_3-sulphate (T_3S) were similar in both animal groups at baseline (table 2). T_3 and T_4 levels decreased significantly in the PCS group at four weeks after surgery, compared with baseline (T_4: 19.8 ± 8.8 to 6.6 ± 3.0 (p=0.004); T_3: 1.0 ± 0.28 to 0.28 ± 0.21 (p=0.004)). In the SHAM group, T_4 levels were also significantly decreased at four weeks after surgery. However, this decrease in T_4 was not as pronounced as in the PCS dogs (for PCS: 6.6 ± 3.0 vs. 17.6 ± 11 for SHAM (p=0.01))(figure 2a, 2b).

Free T_4, reverse T_3 and the T_3/T_4 ratio remained within normal limits in both groups (table 2, figure 2c, 2d). T_4S was significantly increased in PCS dogs at four weeks after surgery, compared with SHAM dogs. In accordance with the decrease in T_3 and unaltered rT_3 levels, the T_3/rT_3 ratio was decreased in the PCS group at four weeks after surgery, compared with SHAM, and the rT_3/T_4 ratio was increased. The T_4S/T_4 ratio was significantly increased, and the T_3S/T_3 ratio was significantly increased in the PCS group at four weeks after surgery, compared with SHAM dogs (table 2).

Table 1 Baseline clinical and biochemical parameters of dogs with a portacaval shunt and partial hepatectomy (PCS) and sham-operated controls (SHAM), compared with levels at four weeks after surgery.

	PCS			SHAM			p-value# PCS vs. SHAM	
parameter	**baseline**	**four weeks after surgery**	**p-value***	**baseline**	**four weeks after surgery**	**p-value***	**baseline**	**four weeks after surgery**
HE grade	0	1 (0 - 4)	0.04	0	0	0.73	1.0	0.02
arterial NH_3 (µmol/L)	34 ± 9.8	239 ± 111	0.004	42 ± 11	33 ± 12	0.75	0.29	0.002
CSF NH_3 (µmol/L)	4.8 ± 2.8	96 ± 39	0.13	4.0 ± 2.4	11 ± 8.2	0.06	0.83	0.003
albumin (g/L)	32 ± 2.0	26 ± 3.7	0.008	30 ± 2.4	28 ± 2.7	0.18	0.33	0.09
bile acids (µmol/L)	9 ± 1.6	228 ± 204	0.008	8.3 ± 1.0	21 ± 20	0.18	0.43	0.002
body weight (kg)	11.5 ± 1.8	9.4 ± 1.4	<0.001	11.5 ± 1.8	10.0 ± 1.5	<0.001	0.49	0.90

Grade hepatic encephalopathy (HE): median (range); all other parameters: mean±SD(n). *p-value: body weight: two sample t test (paired). All other parameters: Wilcoxon two-sided matched pairs signed rank sum test: baseline vs. four weeks after surgery; #p-value: body weight: two sample t test (unpaired). All other parameters: Wilcoxon rank sum test.: PCS vs. SHAM.

Table 2 Thyroid hormones, ratios and metabolites in serum of dogs with a portacaval shunt and partial hepatectomy (PCS) and sham-operated controls (SHAM). Baseline values compared with levels at 4 weeks after surgery.

	PCS			SHAM			p-value# PCS vs. SHAM	
parameter	**baseline**	**four weeks after surgery**	**p-value***	**baseline**	**four weeks after surgery**	**p-value***	**baseline**	**four weeks after surgery**
total T_4 (nmol/L)	19.8±8.8	6.6±3.0	0.004	24.3±10.3	17.6 ±11.0	0.04	0.37	0.01
total T_3 (nmol/L)	1.0±0.28	0.28±0.21	0.004	1.1±0.20	0.61±0.32	0.29	0.29	0.02
free T_4 (pmol/L)	17.7±7.2	18.5±4.3	0.70	21.7±7.4	18.4±4.5	0.39	0.26	0.97
reverse T_3 (pmol/L)	0.6±0.19	0.49±0.15	0.13	0.6±0.16	0.56±0.20	0.36	0.97	0.46
T_4 sulphate (pmol/L)	162±58	253±106	0.02	199±59	251±132	0.67	0.22	0.96
T_3 sulphate (pmol/L)	364±124	305±164	0.46	413±136	276±102	0.13	0.45	0.68
T_3 / T_4 ratio	0.05±0.02	0.05±0.03	0.30	0.05±0.02	0.04±0.03	0.49	0.56	0.88
T_3 / rT_3 ratio	1.9±0.78	0.53±0.28	0.002	2.0±0.40	1.5±0.99	0.45	0.72	0.01
rT_3 / T_4 ratio	0.03±0.01	0.11±0.09	0.04	0.03±0.01	0.04±0.02	0.69	0.21	0.08
T_4S / T_4 ratio	8.7±3.3	53±37.0	0.004	8.7±3.1	14.6±9.9	0.22	0.96	0.01
T_3S / T_3 ratio	348±89	1649±1709	0.004	381±162	487±238	1.0	0.92	0.003

*p-value: total T_4, total T_3, T_4S/T_4 ratio and T_3S/T_3 ratio: two-sided Wilcoxon matched pairs signed rank sum test; all other parameters: two sample t test (paired).: baseline vs. four weeks after surgery. #p-value: total T_3, T_4S/T_4 ratio and T_3S/T_3 ratio: Wilcoxon rank sum test: all other parameters: two sample t test (unpaired): PCS vs. SHAM.

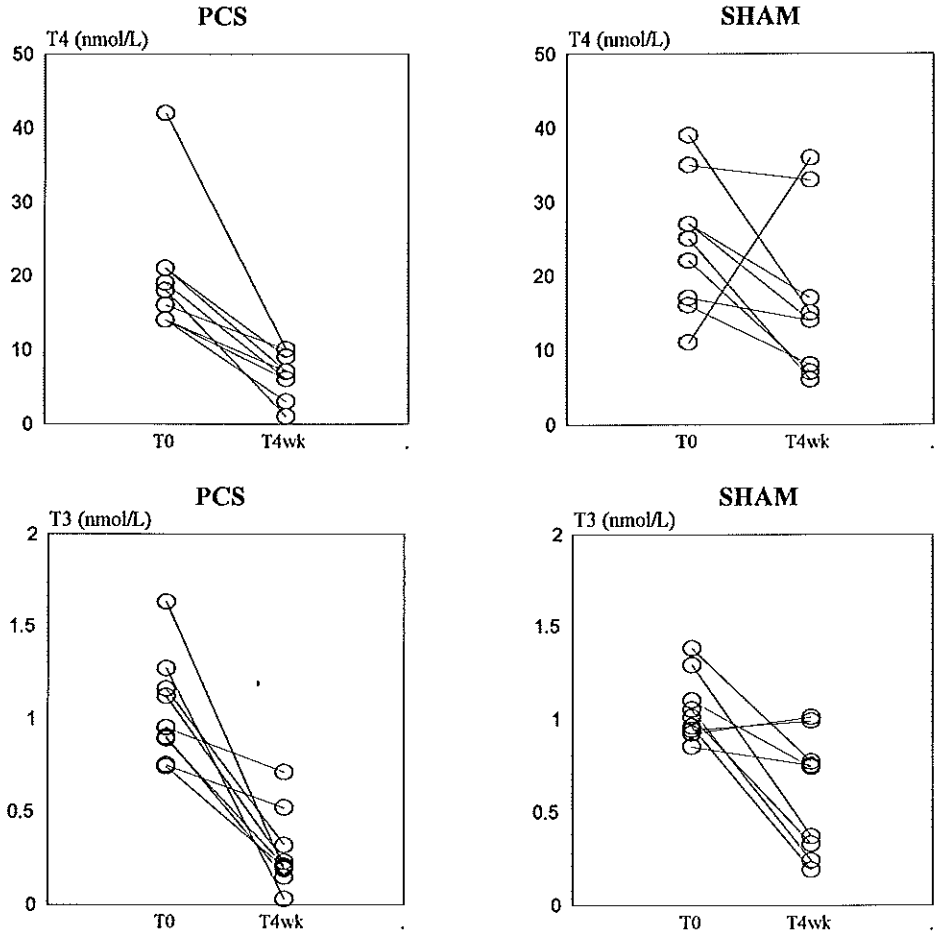

Figure 2a and 2b: T_4 levels and T_3 levels of PCS and SHAM dogs. Baseline values, compared with values at four weeks. Left graph: PCS dogs, right graph: SHAM dogs. X-axis: T0: baseline values; T4 wk: levels at four weeks. Y-axis: T_4 and T_3 levels, expressed in nmol/L.

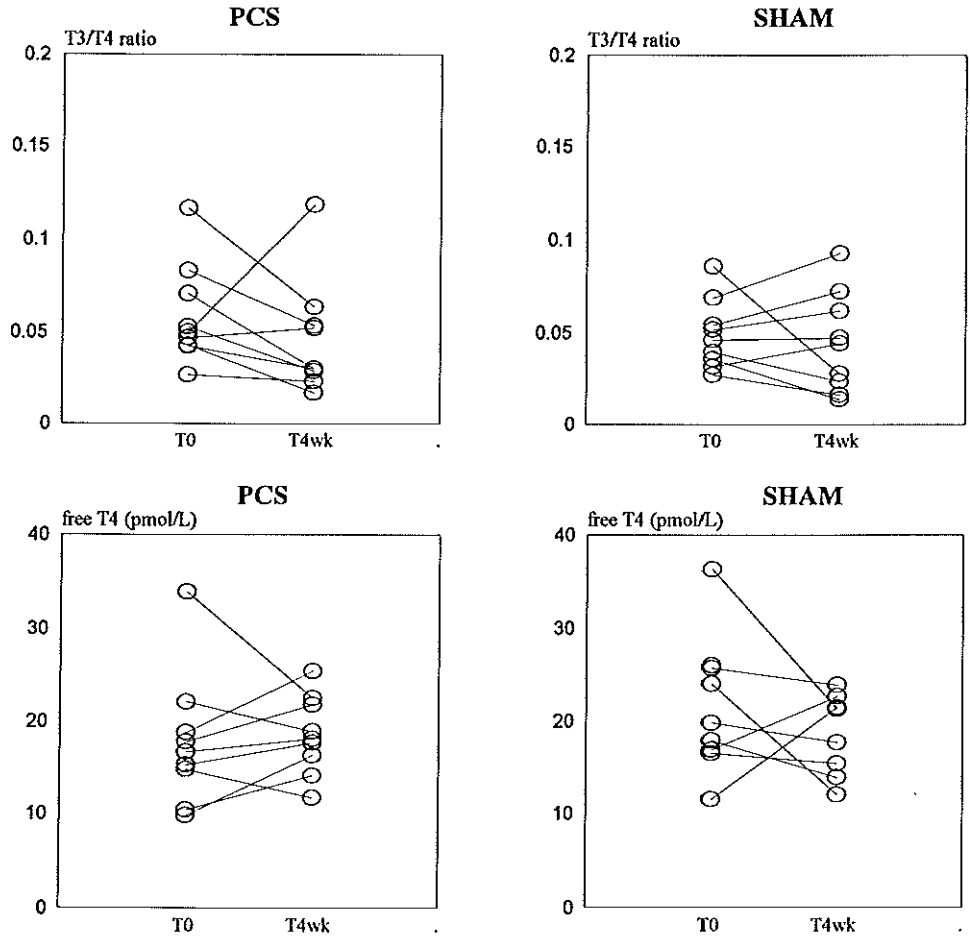

Figure 2c and 2d: T_3/T_4 ratio and free T_4 levels of PCS and SHAM dogs. Baseline values, compared with values at four weeks. Left graph: PCS dogs, right graph: SHAM dogs. X-axis: T0: baseline values; T4 wk: levels at four weeks. Y-axis: free T_4 levels, expressed in pmol/L.

Discussion

Total T_3 and T_4 levels were significantly decreased in dogs with a portocaval shunt and a 40% hepatectomy. Free T_4 remained unaltered four weeks after surgery, indicating that euthyroidism is maintained in serum. In pair-fed SHAM operated dogs, total T_4 and T_3 levels decreased at four weeks after surgery, which was significant for T_4 only.

How can we explain our results ?

Low T_4 and T_3 and normal free T_4 levels indicate decreased plasma protein binding of T_4 and T_3. Decreased plasma binding could be the result of decreased hepatic synthesis of thyroid binding proteins. [1,2] Although thyroid binding proteins were not measured in this study, a decrease in cortisol binding protein has been described in the same animal model. [14] In addition, presence of hypalbuminemia and the partial hepatectomy of PCS dogs supports the notion of diminished hepatic protein synthesis. Moreover, competitive inhibitors of plasma protein binding may have contributed to the low T_4 and T_3 levels. [15]

The liver is the major site of extra-thyroidal T_3 production. In humans, 80 % of T_3 is synthesized in the liver from T_4. [16] Firstly, the low plasma T_3 levels found in PCS dogs could be explained by decreased hepatic synthesis by type 1 iodothyronine deiodinase (D1). Sulphated T_4 and T_3 (T_4S and T_3S) are high affinity substrates for D1, making them sensitive markers for hepatic D1 activity (17). Presence of increased levels of T_4S in PCS dogs in addition to increased ratios of T_4S/T_4 and T_3S/T_3 all indicate diminished hepatic D1 activity.

Secondly, low plasma T_3 levels could be the result of diminished uptake of its precursor T_4 into hepatocytes. Decreased hepatic uptake of T_4 has been described in patients with liver cirrhosis [2], and in other non-thyroidal illnesses and caloric deprivation . [19-21] Specific inhibitors of serum binding of T_3 and T_4 may also compete with T_4 uptake into hepatocytes. Compounds with these inhibitory properties include: bilirubin, nonesterified fatty acids, 3-carboxy-4-propyl-2-furan propanoic acid (CMPF), indoxyl sulphate and L-amino acids. [16,17] Substances that inhibit hepatic uptake of T_4 may also compromise brain uptake of T_4. For example, L-amino acids have shown to competitively inhibit uptake of T_4 into mouse neuroblastoma cells. [18]

SHAM dogs also showed a significant decrease in T_4 and a trend towards a decrease in T_3 levels at four weeks after surgery. As body weight is decreasing significantly also in the SHAM group, this finding is probably the result of caloric deprivation after pair-feeding. [19-21]

How reliable are our findings ?

The animal model we used is a highly reproducible model for chronic liver disease. As a result of the induction of a portal-systemic shunt in combination with a 40 % reduction in liver cell mass, dogs develop hypalbuminemia, hyperammonemia and clinical signs of hepatic encephalopathy within four weeks after surgery . [9]

Our study indicates, that portal-systemic encephalopathy is accompanied by low T_4 and T_3 levels and normal free T_4, which is comparable to findings in humans with chronic liver disease. [2,3]

Probably, these alterations in thyroid hormone metabolism reflect the severity of liver disease and caloric deprivation, like in human non-thyroidal illness. The contribution of altered thyroid hormone metabolism to the pathogenesis of portal-systemic enephalopathy remains speculative. However, T_4 uptake into the brain may be compromised by the same substances that interfere with plasma thyroid hormone binding and with T_4 uptake into the liver. Future research is needed to prove whether altered brain uptake if thyroid hormone into the brain is related to hepatic encephalopathy.

References

1. Kaptein EM. Thyroid hormone metabolism in illness. In: Hennemann G ed. Thyroid hormone metabolism, basic and clinical endocrinology series, volume 8, New York, USA, Marcel-Dekker, 1986:297-333.
2. Sheridon P. Thyroid hormones and the liver. Clinics in Gastroenterology 1983;12:797-818.
3. Green JRB, Snitcher EJ, Mowat NAG. Thyroid function and thyroid regulation in euthyroid men with chronic liver disease: evidence of multiple abnormalities. Clin Endocrinol 1977;7:453-61.
4. Chopra IJ, Hersman JM, Pardridge WM, Nicoloff JT. Thyroid function in nonthyroidal illnesses (UCLA conference). Ann Int Med 1983;98:946-57.
5. Borzio M, Caldara R, Borzio F, Piepoli V, Rampini P, Ferrari C. Thyroid function tests in chronic liver disease: evidence for multiple abnormalities despite clinical euthyroidism. Gut 1983;24:631-36.
6. Caregaro L, Alberino F, Amodio P, Merkel C, Angeli P, Plebani M. Nutritional and prognostic significance of serum hypothyroxemia in hospitalized patients with liver cirrhosis. J Hepatol 1998;28:115-21.
7. Rijt CCD van der. Hepatic encephalopathy: clinical and experimental studies [dissertation]. Rotterdam:Erasmus Univ.;1991.
8. Hitoshi S, Terao Y, Sakuta M. Portal-systemic encephalopathy and hypothalamic hypothyroidism: effect of thyroid hormone on ammonia metabolism (case report). Int Med 1993;32(8):655-58.
9. Meyer HP, Legemate DA, How T, van den Brom WE, Voorhout G, Chamuleau RAFM et al. End-to-side portocaval shunt and partial hepatectomy in the dog. A model for chronic hepatic encephalopathy. Submitted for publication.
10. Rothuizen J, van den Ingh TSGAM, Voorhout G, van der Leur RJT, Wouda W. Congenital porto-systemic shunts in sixteen dogs and three cats. J Small Anm Pract 1982;23:67-81.
11. Eelkman Rooda SJ, Kaptein E, Visser TJ. Serum triiodothyronine sulphate in man measured by radioimmunoassay. J Clin Endocrinol Metab 1989;69:552-56.
12. Wu SY, Huang WS, Polk D, Florsheim WH, Green WL, Fisher DA. Identification of thyroxine sulfate (T_4S) in human serum and amniotic fluid by a novel T_4S radioimmunoassay. Thyroid 1992;2:101-5.
13. Chopra IJ, Wu SY Chua Teco GN, Santini F. A radioimmunoasssay of 3,5,3' - triiodothyronine sulfate: studies in thyroidal and nonthyroidal diseases, pregnancy, and neonatal life. J Clin Endocrinol Metab 1992;75:189-94.
14. Meyer HP, Rothuizen J. Increased free cortisol in plasma of dogs with porto-systemic encephalopathy (PSE). Dom Animal Endocrinol 1994;11:317-22.
15. Chopra IJ, Scolopion DH, Hepner GW, Morgenstein AA. Misleadingly low free thyroxine index and usefulness of reverse triiodothyronine measurements in non-thyroidal illnesses. Ann Intern Med 1979;90:905-12.
16. Hennemann G, Everts ME, De Jong M , Lim C-F, Krenning EP, Docter R. The significance of plasma membrane transport in the bioavailability of thyroid hormone. Clin Endocrinol 1998;48:1-8.
17. Visser TJ. Sulfation and glucuronidation pathways of thyroid hormone metabolism. In: Wu S-Y, Visser TJ Eds.

Thyroid hormone metabolism: molecular biology and alternate pathways. CRC Press, Boca Raton, USA, pp. 85-117.

18. Laksmanan M, Goncalves E, Lessly G, Foti D, Robbins J. The transport of thyroxine into mouse neuroblastoma cells, NB41A3: the effect of L-system amino acids. Endocrinology 1990;126:3245-50.
19. Van der Heyden JTM, Docter R, van Toor H, Wilson JHP, Hennemann G, Krenning EP. Effect of caloric deprivation on thyroid hormone tissue uptake and generation of low-T_3 syndrome. Am J Physiol 1986;251(Endocrinol Metab 14) E156-E163.
20. De Jong M, Docter R, van der Hoek HJ, Vos RA, Krenning EP, Hennemann G. Transport of T_3 into the perfused rat liver and subsequent metabolism are inhibited by fasting. Endocrinology 1992;131:463-70.
21. De Bruijne JJ, Altszuler N, Hampshire J, Visser TJ, Hackeng WHL. Fat mobilization and plasma hormone levels in fasted dogs. Metabolism 1981;30:190-4.

Effect of flumazenil on the electroencephalogram of patients with portal-systemic encephalopathy. Results of a double blind, randomized, placebo-controlled multicenter trial.

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Abstract

The efficacy of the benzodiazepine antagonist flumazenil has been assessed clinically in a double blind, randomized, placebo-controlled multicentre study in patients with grade I-III portal-systemic encephalopathy. In an ancillary study reported here the effect of flumazenil on the electroencephalogram (EEG) was analysed in 32 patients who had EEG-grading according to protocol. Following the baseline observation period, patients were randomized to receive (at one minute interval) three sequential bolus injections of flumazenil (0.4, 0.8 and 1 mg) or placebo, followed by infusions of flumazenil (1 mg/hr) or placebo for 3 hours. Patients were monitored for 5 hours after infusion. A positive response was defined as 1 point improvement in EEG grade. After independent analysis of the EEG gradings five out of 17 (29%) flumazenil treated patients showed an improvement in EEG-grading (3 after bolus, two during follow-up) compared to two out of 15 (13%) placebo treated patients (1 after bolus and during follow-up, 1 during follow-up) (95% confidence interval of difference: -12% to + 50 %). Of the five EEG-responders after flumazenil, three also had an improvement in clinical PSE grading (none after bolus, two during infusion, one during follow-up), compared to neither of the two EEG-responders after placebo. EEG responders did not differ from non-responders with respect to Child-Pugh score, basal EEG, PSE grade and positivity for benzodiazepines. In conclusion, treatment perspectives for flumazenil in portal-systemic encephalopathy appear to be present for only a minority of patients; however, this study yield no support for a major role of benzodiazepine antagonists in the treatment of hepatic encephalopathy.

Introduction

Flumazenil is an imidazobenzodiazepine, which has a high affinity for the benzodiazepine receptor. Flumazenil blocks the central effects of substances that act via the benzodiazepine receptor by competitive inhibition. [1] Intravenous administration of flumazenil promptly reverses the hypnotic-sedative effects of benzodiazepines. [2] Since altered GABA-benzodiazepine neurotransmission and endogenous benzodiazepine-like substances have been implicated in the pathogenesis of portal-systemic encephalopathy (PSE), [3,4,5] flumazenil has been evaluated in open [6-11] and double blind [12-15] clinical studies. Most studies indicate an effect of flumazenil on clinical grades of PSE. The results of an international multicentre study to evaluate the acute effects of intravenous flumazenil on PSE in a group of patients carefully screened negative for benzodiazepine in blood and urine, a positive effect on the clinical PSE grade was observed. [15] We acknowledge the small effect on clinical PSE grading in four double-blind studies, but consider electrophysiologic slowing of brain function a key feature of PSE. Therefore, we performed an ancillary analysis of EEG readings of patients who had these measurements done according to the protocol.

Patients and methods

Patients

A total of 49 patients were enrolled and 28 were randomized to the flumazenil group and 21 to the placebo group. In this ancillary study we analysed all patients with complete EEG readings in the intent-to-treat analysis: 32 of 49 patients initially enrolled in the multi-centre trial were analysed (17 in the flumazenil and 15 in the placebo group).

Only patients with portal-systemic encephalopathy due to chronic liver failure entered the study. Patients with acute hepatic failure, coma (PSE, stage IV), encephalopathy other than due to liver failure, severe gastrointestinal bleeding, acute hepatitis superimposed on cirrhosis, Wilson's disease, hemochromatosis, severe cerebral atrophy (as assessed by cranial Computer Aided Tomography), any psychiatric disease except PSE as well as patients receiving psychotropic medication were excluded from the study. The severity of liver disease was assessed by the Child-Pugh score. [16]

Study design

The duration of the study was 12 hours. It consisted of a four hour baseline observation period, a three hour double blind treatment period and a five hour follow-up period. Following the baseline observation period, patients were randomized to receive (at one minute intervals) three sequential bolus injections of flumazenil (0.4, 0.8, 1 mg) or placebo, after which patients received intravenous infusions of either flumazenil (1 mg/hr) or placebo for three hours. No concomitant therapy was permitted during the baseline and double-blind treatment periods, with the exception of saline and/or glucose, lactulose, potassium and vitamin K. During the post-treatment observation period standard PSE treatment (e.g. antibiotics) was allowed according to the patient's condition. Randomisation was performed according to a computer-generated randomisation list produced by F. Hoffmann-La Roche Ltd, and according to Roche's Standard Operational Procedures. Each clinician and study director received a sealed envelope corresponding to each patient, containing the identity of drug administered to that patient. The double-blind code was broken only after final data cleaning had taken place and after the database had been locked.

Evaluations

The EEG was recorded continuously during the first and the last 20 minutes of the baseline observation period and the double blind period. In the follow-up period EEG was recorded twice within the first two hours. Clinical experience with flumazenil (when used as a reversal agent for exogenously administered benzodiazepines) has shown that its onset of action is generally very rapid [2]. For this reason EEG-gradings were separately analysed comparing the baseline grading (the mean of two recording periods) with the EEG-grading immediately after the bolus injection and the EEG-grading after the end of the infusion period.

To evaluate late effects of flumazenil therapy (as suggested by others [13]), we also compared the baseline EEG grading with the EEG grading from the follow-up period (mean of two recordings). EEG-grading was performed using subscores obtained by visual inspection. [17] EEG grading by visual inspection was performed by one experienced investigator, using the following modification (developed by Scollo-Lavizzari): grade 0: 8-12 sec basic rhythm, mean dominant frequency (MDF) > 8 sec, % theta <20; grade 1: sudden shifts between a normal alpha frequency (around 9-10 sec) and slow substitutes (6-8 sec); MDF >7 sec, % theta >35; grade 2: diffuse slow activity posterior alpha rhythm seen occasionally, MDF 5-7 sec, % theta > 60; grade 3: dominant slow activity in all areas, MDF 3-5 sec, % delta 70; grade 4: bilaterally synchronous, 2 to 3 per second waves, predominating over frontal lobes and spreading backwards to occipital lobes; occasional short-lived appearence of faster rhythms (5 to 6 per second) or voltage depression, MDF < 3 sec, % delta 70. In three flumazenil treated patients and four placebo treated patients (all from one center) the EEG was also graded by automated EEG analysis; [18,19] the results of both gradings were identical. A positive response was defined as 1 point improvement in EEG-grading during treatment or follow-up, compared with the baseline period.
Clinical PSE grading (comprising disorder of sleep pattern (anamnestic), level of consciousness: drowsiness (including mood disorder, inappropriate behaviour, orientation, mental task) , somnolence, stupor and coma [20,22]) was performed at 60-minute intervals during the baseline and post-treatment periods and every 30 minutes during treatment, the first assessment taking place within five minutes of the bolus injections. A positive response was defined as a two-point improvement in clinical PSE grading any time during treatment or follow-up, compared with the baseline period.

Statistical analysis

Demographic and clinical data between flumazenil and placebo treated patients were compared using the Fisher exact test (sex and Child-Pugh score) and Student-t test (age, basal EEG and PSE grade). Mean improvement in EEG grading was analysed using Wilcoxon rank sum test and the percentage improvement in EEG grading was tested, using Fisher exact test, comparing the flumazenil treated patients with placebo treated patients.

Results

Demographic and clinical data (age, sex, basal EEG grade, basal PSE grade and Child-Pugh score) are presented in table 1. These data were not statistically different. The percentage, mean and median improvement in EEG-grading was calculated as indicated in table 2. Figure 1 graphically presents the percentage improvement in EEG grading and the 95 % confidence interval of the difference between the flumazenil and placebo treated group.

Table 1 Demographic and clinical data on flumazenil and placebo group

		Flumazenil (n=17)	Placebo (n=15)	p-value
Age	mean (sd)	53.4 ± 10.1	51.5 ± 9.3	0.76*
	median (range)	57 (32 - 69)	56 (33 - 66)	
Sex	male	15	9	0.15**
	female	2	6	
Basal EEG grade	mean (sd)	3.00 ±1.0	2.65 ± 1.07	0.36*
	median (range)	3.25 (0.50 -4.00)	3.00 (0.75-4.00)	
Basal PSE grade	mean (sd)	6.76 ±2.97	7.27 ± 4.36	0.71*
	median (range)	5.0 (4.0-12.5)	5.0 (3.0-14)	
Child-Pugh score	A	1	1	
	B/C	16	14	0.85**

Data are presented as mean±SD and median with range. *p-value: student-t test; **p-value: fisher exact test

The analysis showed an improvement in 5 out of 17 patients (29%) in the flumazenil treated group and 2 out 15 (13%) in the placebo treated group. In the flumazenil treated group 3 out of 5 patients had an improvement in EEG-grade immediately after bolus injection and the other 2 patients improved during follow-up. Three of them also showed an improvement in PSE score, 2 during the infusion period and one during follow-up. In the placebo treated group two patients had an improvement in EEG-grade: one patient improved after bolus injection and during follow-up (after a deterioration during infusion); the other patient improved during follow-up. None of them improved with respect to their PSE score (table 3).

EEG-responders and non-responders do not differ with respect to their Child-Pugh score, basal EEG and basal PSE grading (data not shown). Although there appears to be a trend for a flumazenil effect, the results show no significant difference in effect at any time during the study between control patients and flumazenil treated patients. At the end of treatment the difference in percentage of response was 16% with a 95% confidence interval of -12% to 50 %.

Table 2 Improvement in EEG grading during and after therapy

		Flumazenil (n=17)	Placebo (n=15)	p-value
baseline EEG vs. EEG after bolus	% (n)	18 (3)	7 (1)	1.0*
	mean (SEM)	0.35 (0.59)	0.25 (0.46)	
	median (range)	0 (-0.75 - 1.5)	0 (-0.5 - 1)	0.38
baseline EEG vs. EEG after infusion	% (n)	18 (3)	0 (0)	0.23
	mean (SEM)	0.35 (0.78)	0.05 (0.37)	
	median (range)	0 (-0.5 - 0.75)	0 (-0.75 - 2)	0.27
baseline EEG vs. EEG after follow-up	% (n)	29 (5)	13 (2)	0.40
	mean (SEM)	0.38 (0.91)	0.22 (0.48)	
	median (range)	0 (-0.75 - 2)	0 (-0.5-1)	0.29

Baseline period comprises two separate EEG recording periods (both gradings averaged); after bolus injection and after infusion period one EEG recording was made; the follow-up period also comprises two EEG recordings (both gradings averaged). *p-value: Fisher exact test; all other p-values: Wilcoxon rank sum test.

Discussion

The results of the double-blind placebo-controlled international multicentre study on the effect of flumazenil on clinical grades of portal-systemic encephalopathy (PSE) have recently been reported. [15] Clinical PSE grading is emerging as the standard method for evaluation of encephalopathy. However, since the components of the PSE index are changing [20], the PSE index should be validated. In other studies on flumazenil in hepatic encephalopathy. [7,10,12-14] EEG recording was included in the efficacy evaluation and the changes in clinical status after flumazenil therapy were reflected in the EEG in most patients. Moreover, in one study [13] early effects of flumazenil therapy on the cerebral status were evident before clinical improvement could be substantiated. EEG grading by spectral analysis is an objective and accurate method to measure the degree of electrophysiological slowing in patients with hepatic encephalopathy. [18,19,20] EEG gradings based on standard recordings and assessed by one experienced observer yields results of equivalent reliability, as EEG by spectral analysis. [21] This ancillary study documents the EEG findings of the flumazenil international multicentre study [15] and combines these with the results of clinical PSE grading. The EEG recordings showed no significant effect of flumazenil on the grade of hepatic encephalopathy, although there was a trend due to an effect in a small group of patients. A strong point for a flumazenil effect in a subgroup of patients is that 3 out of 5 responders also showed an improvement in PSE score. In addition, three flumazenil responders showed an EEG improvement just after bolus injection.

Table 3 Improvement in EEG grading compared to clinical PSE score in patients treated with flumazenil and placebo

	baseline	*treatment*		*follow-up*
		bolus	*infusion*	
Flumazenil				
EEG	4	3	2	2
	2	1	0	0
	4	4	4	2
	3	3	3	2
	3	2	2	2
PSE	11	11	10	10
	4	4	1	1
	5	5	5	1
	8	8	8	9
	4	3	0	0
Placebo				
EEG	4	4	4	3
	3	2	3	2
PSE	12	11	11	11
	4	4	4	4

Baseline: the average of two EEG recording periods and the average of the first and last PSE score; bolus: EEG/PSE just after bolus injection; infusion: EEG/PSE after the end of the infusion period; follow-up: the average of two EEG recording periods and the average of the first and last PSE score.

How reliable are our findings? No doubt the number of patients in the study was too small for an answer with a small confidence interval. In practice, it turned out that several centers had great difficulty to register EEG in an appropriate manner at times outside normal office-hours. In view of the low recruitment, it was decided that the EEG was no longer mandatory as originally planned, but only optional. Therefore only 32 patients from the recruited 49 patients got complete EEG recordings; this number was less than the planned total sample size of 40 patients. The power of the study might therefore be insufficient to show efficacy based on EEG alone. Other factors which may affect the outcome are the methods of recording EEG's, the multicentre nature of the study and the fact that the analysis was by intent-to-treat and not matched by a per-protocol analysis. The outcome of this study is, however, in agreement with the 4 double blind placebo controlled studies reporting a clinical effect of flumazenil in a small proportions of patients. [12-15]

How to explain the effect of flumazenil in a small proportion of patients? Reversal of the effect of endogenous benzodiazepine has been suggested. [4,23] Recent work on the levels of benzodiazepines in cirrhosis without or with encephalopathy in comparison to exogenous benzodiazepine users, however, suggest that endogenous levels of benzodiazepine in cirrhosis with encephalopathy are

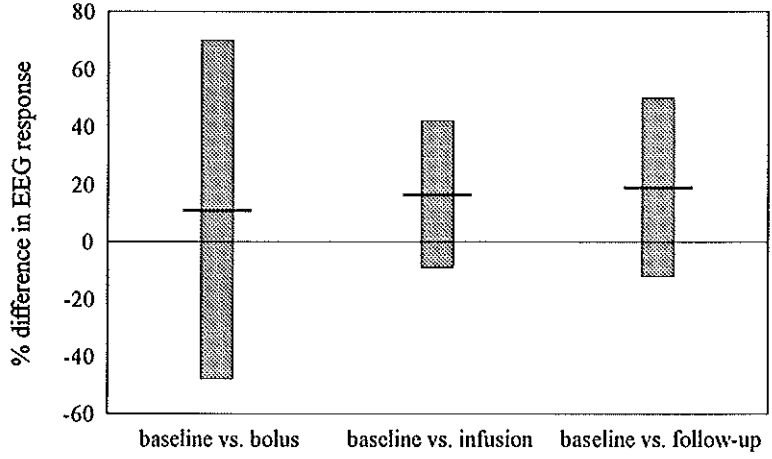

Figure 1 Percentage difference in EEG response (including 95 % CI of difference) between flumazenil and placebo treatment.

far below levels observed in exogenous drug users without encephalopathy. [24] Therefore, it remains possible that the effect of flumazenil should be explained by reversal of an inapparent exogenous benzodiazepine with a long half life. Slowing of electrophysiologic brain activity - as measured by EEG - is a key characteristic of all patients with portal-systemic encephalopathy. Since the effect of flumazenil on EEG is limited to a minority of patients, this study yields no support for a major role of benzodiazepine antagonists in the pathogenesis of hepatic encephalopathy. In addition, these findings indicate, that activated benzodiazepine neurotransmission is of no major relevance to the pathogenesis of hepatic encephalopathy. Other studies in animals without potential contamination by exogenous benzodiazepine have come to a similar conclusion. [25, 26]

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References

1. Haefely W. The preclinical pharmacology of flumazenil. Eur J Anesthes 1988: Suppl 2: 25-36.
2. Amrein R, Hetzel W, Hartmann D, Lorscheid T. Clinical pharmacology of flumazenil. Eur J Anaesthes 1988: Suppl 2: 65-80.
3. Schafer DF, Jones EA. Hepatic encephalopathy and the γ-aminobutyric-acid neurotransmitter system. Lancet 1982;1:18-20.
4. Mullen KD, Szauter KM, Kaminski-Russ K. "Endogenous" benzodiazepine activity in body fluids of patients with hepatic encephalopathy. Lancet 1990;1:81-3.
5. Basile AS, Hughes RD, Harrison PM, Murata Y, Pannell L, Jones EA, Williams R, Skolnick P. Elevated brain concentrations of 1,4-benzodiazepines in fulminant hepatic failure. N Eng J Med 1991;325:473-8.
6. Bansky G, Meier PJ, Ziegler WH, Walser H, Schmid M, Huber M. Reversal of hepatic coma by benzodiazepine antagonist (Ro 15-1788). Lancet 1985;1:1324-5.
7. Bansky G, Meier PJ, Riederer E, Walser H, Ziegler WH, Schmid M. Effects of the benzodiazepine receptor antagonist flumazenil in hepatic encephalopathy in humans. Gastroenterology 1989;97:744-50.
8. Grimm G, Ferenci P, Kazenschlager R, Madl C, Schneeweiss B, Laggner AN, Lenz K, Gangl A. Improvement of hepatic encephalopathy treated with flumazenil. Lancet 1988;2:1392-4.
9. Meier R and Gyr K. Treatment of hepatic encephalopathy (HE) with the benzodiazepine antagonist flumazenil: a pilot study. Eur J Anaesthes 1988: Suppl 2: 139-46.
10. Scollo-Lavizarri, Steinmann E. Reversal of hepatic coma by benzodiazepine antagonist (Ro 15 -1788). Lancet 1985;1:1324.
11. Ferenci P, Grimm G, Meryn S, Gangl A. Successfull long-term treatment of portal-systemic encephalopathy by the benzodiazepine antagonist flumazenil. Gastroenterology 1989;96:240-3.
12. Pomier-Layrargues G, Giguère, Lavoie J, Perney P, Gagnon S, D'Amour M, Wells J, Butterworth RF. Flumazenil in cirrhotic patients in hepatic coma: a randomized double-blind placebo-controlled crossover trial. Hepatology 1994;19:32-7.
13. Cadranel JF, El Younsi M, Pidoux B, Zylberberg P, Benhamou Y, Valla D, Opolon P. Immediate improvement of hepatic encephalopathy (HE) in cirrhotic patients by flumazenil. Results of a double-blind cross-over study. J Hepatol 1991;Suppl 13:S104.
14. Van der Rijt CCD, Schalm SW, Meulstee J, Stijnen Th. Flumazenil therapy for hepatic encephalopathy: a double-blind cross-over study. Hepatology 1989;10:A85.
15. Gyr K, Meier R, Häussler, Boulétreau P, Fleig WE, Gatta A et al. Evaluation of the efficacy and safety of flumazenil in the treatment of portal systemic encephalopathy: a double blind, randomised, placebo controlled multicentre study. Gut 1996;39:319-24.
16. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646-9.
17. Kennedy J, Parbhoo SP, MacGillivray B and Sherlock S. Effect of extracorporeal liver perfusion on the electroencephalogram of patients in coma due to acute liver failure. Q J Med 1973;42:549-61.
18. Van der Rijt CCD, Schalm SW, de Groot GH, de Vlieger M. Objective measurement of hepatic encephalopathy by means of automated EEG analysis. Electroenceph Clin Neurophysiol, 1984;57:423-6.
19. Van der Rijt CCD, Schalm SW. Spectral analysis in hepatic encephalopathy. In: Bengtsson F, Jeppsson B, Almdal T, Vilstrup H eds. Progress in hepatic encephalopathy and metabolic nitrogen exchange. Florida, CRC Press, 1991:59-66.
20. Conn HO. Quantifying the severity of hepatic encephalopathy. In: Conn HO, Bircher J eds. Hepatic

Encephalopathy. Syndromes and therapies. Medi-Ed Press, Illinois, 1994:13-26.

21. Weissenborn K, Scholz M, Hinrichs H, Wiltfang J, Schmidt FW, Künkel H. Neurophysiological assessment of early hepatic encephalopathy. Electroencephal Clin Neurophysiol 1990;75:289-95.
22. Jones EA, Gammal SA. Hepatic encephalopathy. In: Arias IM, Jakoby WB, Popper H, Schachter D, Shafritz DA editors. The liver: Biology and Pathobiology. Raven Press, New York, 1988:985-1005.
23. Meier R , Gyr K, Scholer A. Persisting benzodiazepine metabolites responsible for the reaction to the benzodiazepine antagonist flumazenil in patients with hepatic encephalopathy. Gastroenterology 1991;101:274-5.
24. Zeneroli ML, Venturini I, Avallone R, Ardizzone G, Baraldi M. Endogenous benzodiazepine in serum of liver cirrhosis patients. J Hepatol 1993;Suppl 1:S188-89.
25. Widler P, Fisch HU, Schoch P, Zimmermann A, Schläpfer TE, Reichen J. Increased benzodiazepine-like activity is neither necessary nor sufficient to explain acute hepatic encephalopathy in the thioacetamide-treated rat. Hepatology 1993;18:1459-64.
26. Van der Rijt CCD, de Knegt RJ, Schalm SW, Terpstra OT, Mechelse K. Flumazenil does not improve hepatic encephalopathy associated with acute ischemic liver failure in the rabbit. Metabol Brain Dis 1990;5:131-41.

Part 2

Studies on subclinical hepatic encephalopathy

The clinical significance of 12 hour electroencephalography in patients with liver cirrhosis and (sub)clinical hepatic encephalopathy.

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Abstract

Two decades ago worsening of the electroenecephalogram (EEG) was reported in cirrhotic patients after meals. In addition, patients with subclinical hepatic encephalopathy sometimes report functional impairments in the second part of the day. Therefore, 12 hour portable EEG registration and repetitive analysis of the EEG was performed in patients with cirrhosis to evaluate the variability in electrophysiologic slowing and the effect of meals and physical exercise. EEG recordings were also graded by visual inspection.

Twenty-seven patients (5 without encephalopathy; 12 with subclinical hepatic encephalopathy (SHE); 10 with clinically manifest hepatic encephalopathy (HE)) formed the study group.The mean dominant frequency (MDF) was 8.2 Hz (95 % CI: 7.0 - 9.3) for patients without encephalopathy, 7.4 Hz (6.9 - 7.9) for patients with subclinical hepatic encephalopathy, and 4.9 Hz (4.1 - 5.6) for those with clinically manifest encephalopathy. Percentage theta was 17.4 (95 % CI: 10.6 - 24.2), 38.4 (29.6 - 47.2) and 33.2 (26.9 - 39.5), respectively. No significant differences in MDF and percentage theta activity were observed during the morning, after lunch, after exercise, or after dinner. On the basis of visual inspection slowing of the EEG was observed after lunch in 83 % of patients with subclinical hepatic encephalopathy and in 50 % of patients with clinically manifest HE. We conclude that assessment of patients with cirrhosis for hepatic encephalopathy by EEG is hardly influenced by time of the day, meals or exercise, when assessed by mean dominant frequency and percentage theta activity. EEG grading by visual inspection appears a more sensitive method to detect variability in electrophysiologic slowing; however, changes in consciousness during the day reported by patients with cirrhosis rather reflect physiological variability than aggravation of hepatic encephalopathy.

Introduction

The clinical syndrome of hepatic encephalopathy (HE) is characterized by disturbance of mental state and neuromuscular function. In clinical practice, hepatic encephalopathy is classified in four grades. [1] Slowing of the electroencephalogram (EEG) correlates well with the clinical degree of hepatic encephalopathy. [2,3] Slowing of the EEG has also been observed in about 20 % of cirrhotic patients without clinical signs of hepatic encephalopathy; these patients are defined as having subclinical hepatic encephalopathy (SHE). [4]

Patients with SHE often report functional impairments, which appear to be related to certain periods of the day (e.g. after a hot meal, physical exercise). [5] About two decades ago slowing of the EEG was observed in cirrhotics after a meal, independent of the protein content . [6] In routine clinical care, automated EEG analysis is performed at random hours during the day. Therefore, to evaluate the variability of the EEG during the day and the effect of meals and physical exercise, we performed an observational study, using 12 hour portable EEG registration and repetitive analysis of the EEG in patients with cirrhosis.

Patients and methods

Subjects

From January 1995 to May 1, 1997 twenty-seven patients from the department of Hepatogastro- enterology of the University Hospital Rotterdam participated in the study. Inclusion criteria were: histologically proven cirrhosis of the liver, age between 16 and 80 years and written informed consent. Exclusion criteria were: unstable liver disease (e.g. active variceal bleeding, or severe clinical hepatic encephalopathy (grade 3 to 4), history of recent (less than 6 weeks) alcohol abuse, use of benzodiazepines, anti-epileptics, or other psychotropic drugs, inability to perform the psychometric tests (due to insufficient knowledge of the Dutch language or bad vision), and severe medical problems such as congestive heart failure, pulmonary disease, etc. that may influence the clinical and electrophysiological assessment of hepatic encephalopathy.

Assessment

Categorizing patients. Methods used for categorizing patients with clinically manifest hepatic encephalopathy, subclinical hepatic encephalopathy (SHE) and no enephalopathy have been described in detail elsewhere. [3-5,8] Clinically manifest hepatic encephalopathy (HE) was assumed to be present if at least 2 of the following abnormalities were detected: inverted sleep pattern, impaired calculation, disturbed memory, and slowness of speech, or one abnormality in association with a flapping tremor. [8] Subclinical hepatic encephalopathy (SHE) was defined, as the presence of abnormal slowing of the EEG and/or at least one abnormal psychometric test: Number Connection Test part A (NCT-A) or Digit Symbol Test (DST). [4]

The day before the experiment the severity of the liver disease was assessed using the Child-Pugh score. [7] Arterial ammonia levels were measured at screening (the day before the experiment) and on the morning of the day of the experiment. The mean of both measurements was used in the final analysis.

Electroencephalography. Between 8.00 and 9.00 AM of the day of the experiment, electrodes were attached to the skin, and leads T3-O1, T4-O2, C0-O1, C0-O2, F4-C4, C4-P4, F3-C3 and C3-P3 were recorded. [9] Electrode impedance was kept lower than 5kΩ. At regular intervals of 30 minutes and 60 minutes, the patient was asked to lay comfortably in his bed with his eyes closed. Periods of 120 seconds were subsequently marked on the portable EEG recorder. After completion of the 12 hour EEG recording, all marked periods were visually checked by an EEG technician to evaluate the technical quality. Artefact free recordings were selected and spectral analysis of leads T3-O1, T4-O2, C0-O1 and C0-O2 was performed. After applying the usual bandpass filters (0.53 - 35 Hz) recordings of 100 seconds each entered into a computer.

After AD conversion (sample frequency 102.4 Hz), ten epochs of 10 seconds each were analysed by applying Fast Fourier Transformation and the power spectrum was calculated, with expression of the mean dominant frequency (MDF) and the relative theta activity . [3]
The EEG tracings used for spectral analysis were printed and graded by visual inspection by two expert neurophysologists (authors JM and MS-B), using the criteria of Parsons-Smith et al. [2]
Worsening of the EEG was defined present, if the EEG grade increased with at least one grade.
The chronological order of the EEG recordings was changed before visual inspection, and the neurophysiologists were blinded for the spectral analysis results and for the clinical grade of the patient. Both neurophysiologists compared their gradings afterwards, and a final grading was given.

Data analysis and statistics

Patients included in the study were devided into three groups, on the basis of the screening the day before the experiment: group A: patients without HE; group B: patients with SHE; group C: patients with clinically manifest HE.
On the basis of spectral analysis of the EEG the mean dominant frequency (MDF) and percentage theta waves were calculated at the following time points: baseline (10:00 AM); two hours after baseline (at noon), lunch (at 2.00 PM), physical exercise (at 4.30 PM) and dinner (at 6.00 PM).
On the basis of visual inspection, the severity of HE was graded at the same time points.
The mean MDF and percentage theta waves was calculated for each group separately, and equality of medians between baseline values and each of the time points was tested with the Wilcoxon matched-pairs signed-ranks test. The mean difference of the MDF and of the percentage theta activity between baseline and each of the other time points was calculated. The null hypothesis that each median difference equals zero was tested with the Wilcoxon matched-pairs signed-ranks test.

Results

Baseline parameters are listed in table 1. Five patients without HE, twelve patients with SHE and ten patients with clinically manifest HE entered the study. Marked differences in age, sex, etiology of liver disease, or dietary protein content were not observed between the 3 patient groups, although they differed in the severity of liver disease, as reflected by the Child-Pugh score (percentage of patients with Child-Pugh B/C cirrhosis in group A: 60 %, group B: 75 %; group C: 100 %) and arterial ammonia levels.

In figure 1a the mean dominant frequency (MDF) is presented.The MDF was 8.2 Hz (95 % CI: 7.0 - 9.3) for patients without HE, 7.4 Hz (6.9 - 7.9) for patients with SHE, and 4.9 Hz (4.1 - 5.6) for those with clinically manifest HE. In figure 1b the difference in MDF between baseline and the other four time points is presented.

Table 1 Baseline parameters of patients without HE, patients with SHE and patients with clinically manifest HE.

parameter		no HE (n=5)	SHE (n=12)	HE (n=10)
age (median [range])		55 (44 - 58)	50 (29 - 68)	61 (35 - 75)
sex (n)	male	4	7	8
	female	1	5	2
etiology (n)	viral	3	3	3
	alcohol	2	3	4
	other	0	6	3
Child-Pugh score (n)	A	2	3	0
	B/C	3	9	10
arterial ammonia (μmol/L) (mean ± SD)		70 ± 27	88 ± 22	113 ± 45
dietary protein (gr/24 hrs) (mean ± SD)		104 ± 24	80 ± 23	75 ± 15

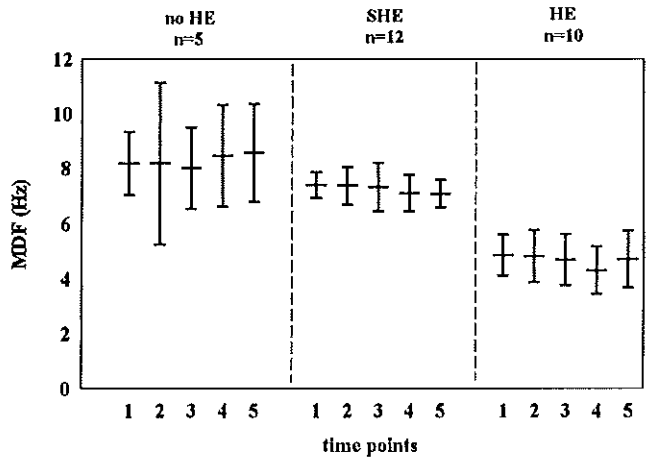

Figure 1a Mean dominant frequency (MDF) of the electroencephalogram by spectral analysis of patients without HE, patients with SHE and patients with clinically manifest HE, measured over 12 hours. X-axis: 1: baseline value; 2: value at 12:00 (noon); 3: value after lunch (2:00 PM); 4: value after exercise (4.30 PM); 5: value after dinner (6:00 PM). Y-axis: mean dominant frequency in Hz ± 95 % confidence interval of mean.

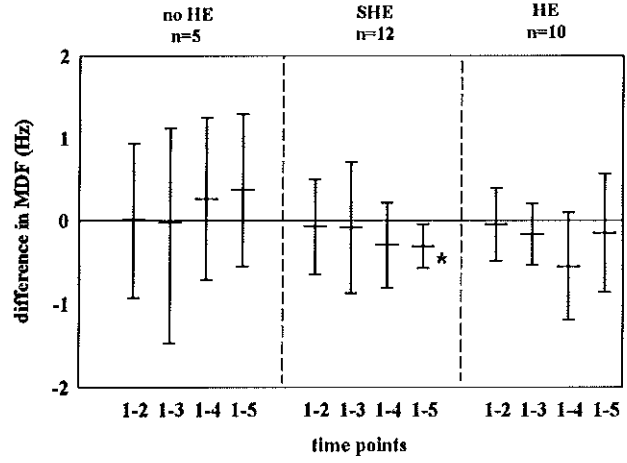

Figure 1b Difference in mean dominant frequency (MDF) of the electroencephalogram by spectral analysis of patients without HE, patients with SHE and patients with clinically manifest HE. Baseline value versus values at noon, after lunch, exercise and after dinner, respectively. X-axis: 1-2: baseline - noon; 1-3: baseline - value after lunch (2:00 PM); 1-4: baseline - value after exercise (4.30 PM); 1-5: baseline - value after dinner (6:00 PM). Y-axis: difference in mean dominant frequency ± 95 % confidence interval of mean difference.

The results indicate, that there is a trend towards a decrease in MDF during the day. However, the mean difference was below zero only after dinner in patients with SHE (-0.31 (-0.57 - -0.04) (p=0.04)), considered not statistically significant in view of multiple testing. In patients with clinically manifest HE the decrease in MDF is largest after lunch, this was also not statistically significance.

In figure 2a the percentage theta waves is presented; the percentage theta was 17.4 (95 % CI: 10.6 - 24.2) for patients without HE, 38.4 (29.6 - 47.2) for patients with SHE and 33.2 (26.9 - 39.5) for those with clinically manifest HE, respectively. No statistically significant changes were found during the day. In figure 2b the difference in percentage theta waves between baseline and the four time points is presented.

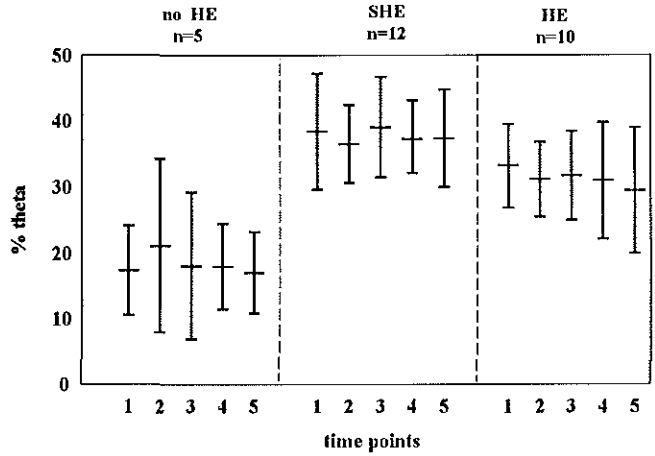

Figure 2a Difference in percentage theta waves of the electroencephalogram by spectral analysis of patients without HE, patients with SHE and patients with clinically manifest HE. Baseline value versus values at noon, after lunch, exercise and after dinner, respectively. X-axis: 1: baseline value; 2: value at 12:00 (noon); 3: value after lunch (2:00 PM); 4: value after exercise (4.30 PM); 5: value after dinner (6:00 PM). Y-axis: mean dominant frequency in Hz ± 95 % confidence interval of mean difference.

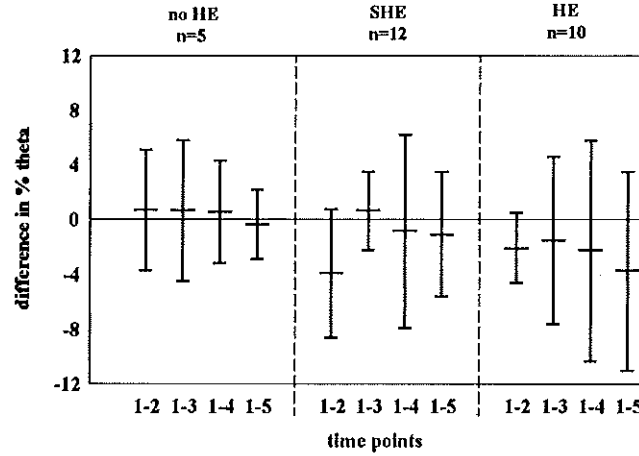

Figure 2b Difference in percentage theta activity of the electroencephalogram by spectral analysis of patients without HE, patients with SHE and patients with clinically manifest HE. Baseline value versus values at noon, after lunch, exercise and after dinner, respectively. X-axis: 1-2: baseline - noon; 1-3: baseline - value after lunch (2:00 PM);1-4: baseline - value after exercise (4.30 PM); 1-5: baseline - value after dinner (6:00 PM). Y-axis: difference in mean percentage theta waves ± 95 % confidence interval of mean difference.

Table 2 shows the number of patients in each group with worsening of the EEG by visual inspection. On the basis of visual inspection of the EEG, worsening of the EEG was observed after lunch in 83 % of patients with SHE and in 50 % of patients with clinically manifest HE. EEG grade reverted to baseline in the majority of patients. However, in 4 out of 10 patients with SHE, and in 2 out of 5 patients with clinically manifest HE slowing of the EEG remained present throughout the afternoon; no additional slowing of the EEG was observed in these patients after exercise or after dinner.

Table 2 Worsening of EEG tracings by visual inspection of patients without HE (A), patients with SHE (B) and patients with clinically manifest HE (C) at entry.

	worsening of EEG (n (%))			
	baseline - 12:00	baseline - 2:00 PM	baseline - 4:30 PM	baseline - 6:00 PM
HE by visual inspection (n / %)				
no HE (n=5)	1 (20 %)	0 (0 %)	1 (10 %)	1 (10 %)
SHE (n=12)	3 (25 %)	10 (83 %)	4 (30 %)	4 (30 %)
HE (n=10)	1 (10 %)	5 (50 %)	2 (20 %)	3 (30 %)

Discussion

Continuous electroencephalography in patients with cirrhosis showed no significant slowing of the EEG during the day, when assessed by the mean dominant frequency and percentage theta waves; on the basis of visual inspection, worsening of the EEG grade after lunch was observed in patients with SHE and HE.

Our findings indicate, that grading by visual inspection is a more sensitive method to detect electrophysiologic slowing during the day. The changes apparently are subtle as they are not reflected in significant variability in MDF nor in percentage theta waves, when assessed by spectral analysis. The high sensitivity of subjective grading by visual inspection of the EEG may have influenced the results of past studies on prolonged EEG recordings. [6]

We used the method of spectral analysis of the EEG, which was validated in our department more than 10 years ago as an objective tool to quantify electrophysiologic slowing in hepatic encephalopathy. [3,4,10]

The method yields erroneous results if insufficient attention is given to technical aspects and interfering artefacts; if technical adequate tracings are analyzed results are reproducible and congruent to grading by visual inspection. [10] Furthermore, we assessed the variability of the over time in three groups of patients with varying grades of encephalopathy, and our results fit those reported by others. [11]

What are the implications of this study ?

Since we adhere greater importance to changes in MDF and percentage theta waves than to subtle changes detected by visual inspection, we think that changes in consciousness during the day reported by patients with cirrhosis are within the physiological range and not aggravation of hepatic encephalopathy by meals or exercise. Our results also imply that assessment of hepatic encephalopathy can be done at random hours during the day.

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References

1. Conn HO. The hepatic encephalopathies. In: Conn HO, Bircher J, eds. Hepatic encephalopathy: syndromes and therapies. Bloomington: Medi-Ed press; 1994. P. 1-12.
2. Parsons-Smith BG, Summerskill WHJ, Dawson AM, Sherlock S. The electroencephalopgraph in liver disease. Lancet 1957;2:867-1.
3. Van der Rijt CCD, Schalm SW, de Groot GH, de Vlieger M. Objective measurement of hepatic encephalopathy by means of automated EEG analysis. Electroencephalopgraph Clin Neurophysiol 1984;57:423-26.
4. Quero JC, Hartmann IJC, Meulstee J, Hop WCJ, Schalm SW. The diagnosis of subclinical hepatic encephalopathy in patients with cirrhosis using neuropsychological and automated electroencephalogram analysis. Hepatology 1996;24:556-60.
5. Groeneweg M, Quero JC, de Bruijn I, Hartmann IJC, Essink-Bot ML, Hop WCJ, Schalm SW. Subclinical hepatic encephalopathy impairs daily functioning. Hepatology 1998;28:45-49.
6. Van Leusen R. Results of protein and non-protein loading tests in patients with hepatocerebral intoxication. Digestion 1972 (suppl);45:244-5.
7. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646-9.
8. Van der Rijt CCD, Mulder P, Krenning EP, Schalm SW. Encephalopathy in liver disease: potential pathogenetic factos identified by logistic regression analysis. Submitted for publication.
9. Jasper HH. The ten -twenty electrode system of the international federation. Electroenceph Clin Neurophysiol 1958; 10: 371-375.
10. Groeneweg M, Gyr K, Amrein R, Scollo-Lavizzari G, Williams R, Yoo JY, Schalm SW. Effect of flumazenil on the electroencephalogram of patients with portosystemic encephalopathy. Results of a double blind, randomised, placebo-controlled multicentre trial. Electroencephalograph Clin Neurophysiol 1996;98:29-34.
11. Laidlaw J, Read AE. The E.E.G. in hepatic encephalopathy. Clin Sci 1963;24:109-120.

Subclinical hepatic encephalopathy impairs daily functioning.

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Abstract

Subclinical hepatic encephalopathy (SHE) is assumed to have a negative effect on patients' daily functioning, therefore treatment is recommended. However, no studies have been performed that document the clinical relevance of SHE. We performed a study in which the prevalence of SHE was determined in 179 outpatients with liver cirrhosis using two psychometric tests (Number Connection Test part A, and the Digit Symbol Test) and automated analysis of the electroencephalogram (EEG). SHE was defined by the presence of at least one abnormal psychometric test and/or abnormal slowing of the EEG. The influence of liver cirrhosis and SHE on patients' daily functioning was assessed using the Sickness Impact Profile (SIP) questionnaire. The distribution of SIP scores of the patients with liver cirrhosis differed from the reference scores of the general population. Patients with cirrhosis and SHE (n=48) reported significantly more impairment in all 12 scales of the SIP, in the psychosocial subscore, the physical subscore, as well as in the total SIP score, compared to cirrhotic patients without SHE (n=131). Multivariate analysis taking into account severity of liver disease (Child-Pugh score), presence of varices and alcoholic etiology showed that SHE independently was related to a diminished total SIP score. The reproducibility of the SIP was high when the test was repeated after a 3-month period. We conclude that SHE implies impaired daily functioning and warrants attempts at treatment.

Introduction

Clinical manifestations of hepatic encephalopathy include a decreased intellectual function, personality disorders, an altered level of consciousness and neuromuscular dysfunction [1]. In addition to clinical manifest hepatic encephalopathy[2] , a subclinical stage has been described, which cannot be detected through global clinical examination, but requires specific neuropsychological and neurophysiological examination. [3-12] The prevalence of subclinical hepatic encephalopathy (SHE) is estimated to vary from 30% to 84% according to recent studies using appropriate methods. [12-16] This variation in reported prevalence depends on the kind (psychometric or electrophysiological) and number of tests used, and the population (etiology and severity of the liver disease) tested. [17]

SHE is considered to be clinically relevant for two reasons. First, it could be a preceding stage of clinical manifest hepatic encephalopathy. [3] However, this assumption is not proven, because only one follow-up study [16] supports this concept. Second, the psychomotor deficits found in SHE could have a disadvanting influence on patients' daily functioning, for example in driving a car or at work.[18] In view of the reported high prevalence of SHE and its presumed negative effect on daily life, routine screening of cirrhotic patients for SHE, and treatment of SHE is

recommended. [19-21] However, the need of treatment of SHE is questionable, as a recent study has shown that patients with SHE did not perform worse driving 'on the road' than patients without SHE. [22] Driving a car is an important, but small part of the total spectrum of daily activities. Therefore, on the basis of this study, no conclusions can be made about the effect of SHE on other aspects of daily functioning.

The influence of chronic diseases on daily life can be assessed using 'Health-related Quality of life' questionnaires. [23] The 'Sickness Impact Profile' (SIP) questionnaire [24,25], is such an instrument for overall health assessment and has been used before to determine the influence of chronic liver disease on patients' daily functioning. [26-29]

The aim of the present study was to determine the prevalence of neuropsychological and neurophysiological defects in stable cirrhotic patients attending an university hospital outpatient clinic using two neuropsychological tests and the neurophysiological method of automated analysis of the electroencephalogram (EEG). The SIP questionnaire was used to determine the influence of SHE on daily functioning. In addition, the reproducibility of the SIP in cirrhotic patients was determined.

Patients and methods

Subjects and Investigations

From January 1, 1992 to May 1, 1996 202 consecutive patients attending the outpatient clinic of Hepatogastroenterology of the University Hospital Rotterdam were screened for SHE. Inclusion criteria were: histologically proven cirrhosis of the liver and age between 16 and 80 years. 193 of 202 patients fitted the inclusion criteria; 3 patients had a surgically constructed portacaval shunt (no cirrhosis) and 6 patients only had clinical signs of cirrhosis at screening. Exclusion criteria were: clinical manifest hepatic encephalopathy *, history of recent (less than 6 weeks) alcohol abuse, use of benzodiazepines, anti-epileptics, or other psychotropic drugs, inability to perform the psychometric tests and to complete the SIP questionnaire (due to either insufficient knowledge of the Dutch language or bad vision), and severe medical problems such as congestive heart failure, pulmonary disease, neurologic desease etc. that influence the quality of life measurement.

*Note: Clinically manifest hepatic encephalopathy was assumed to be present if at least 2 of the following abnormalities: inverted sleep pattern, impaired calculation, disturbed secundary memory, and slowness of speech were detected, or one abnormality in association with a flapping tremor.[30] Impaired calculation was assessed by asking the patient to subtract serial sevens from 100 and to repeat a series of six nonconsecutive numbers in the same order (a normal individual can easily remember seven numbers forwards). Memory was tested by asking the patient to remember three objects and to repeat these several minutes later, and by asking the patient about past prime ministers, dates of wars, and events that affect everyone.

In the final analysis, a total of 179 patients with histologically proven cirrhosis of the liver were included. 14 patients were excluded because of incomplete SHE screening and/or inability to fill out the quality of life questionnaire. All patients underwent a clinical and laboratory investigation, psychometric tests, and automated EEG analysis. In addition, the SIP questionnaire was administered. All examinations took place on the same day. Test-retest reliability of the SIP was assessed by re-administering the questionnaire to patients without signs of decompensated liver disease (i.e. jaundice, ascites, encephalopathy, or variceal bleeding) after a 3-month period.

Clinical and Laboratory Assessment.

The Child-Pugh score was used to assess the severity of liver disease. [31] Three biochemical variables (serum albumin, bilirubin and prothrombin time) in addition to the two clinical characteristics (presence or absence of ascites and clinical signs of encephalopathy) determine the Child-Pugh score. Each variable is given one to three points, leading to scores ranging from 5 (excellent liver function) to 15 points (poor liver function). Presence of esophageal or gastric varices was evaluated, using reports of endoscopic investigations in 174 patients, and esophageal X-rays in 5 patients. Alcoholic etiology of liver cirrhosis was defined, as: presence of severe alcohol abuse in patient history, and absence of other etiologic factors.

Neuropsychological Assessment

Number Connection Test part A (NCT-A). This test is a derivative from the Trail Making Test [32] and measures cognitive motor abilities. In the NCT- A patients have to connect numbers printed on paper consecutively from 1 to 25. Age-related normal values of this NCT-A have been developed in 681 persons without liver disease.[33] Normal values are expressed as the mean ± 2 standard deviations. After explanation, abbreviated demonstration tests were administered to be sure the patient had understood it. Errors were not enumerated, but patients were instructed to return to the preceding correct number and then carry on. The test score is the time the patient needs to perform the test, including the time needed to correct the errors. A low score indicates a good performance.

Digit Symbol Test (DST). This is a subtest of the Wechsler Adult Intelligence Scale (WAIS) and measures motor speed and accuracy.[34] The patient is given a list of digits from 1 to 9 associated with symbols and is asked to fill in blanks with symbols that correspond to each number. The test score is the total number of correct sequential matchings of symbols to numbers in a 90-second interval. The normative data used are expressed as percentiles. After explanation of each test, an abbreviated demonstration was administered to ensure that the patient understood the test correctly. Data of 2169 Dutch and Belgian healthy controls serve as a reference group in The Netherlands.[35] A test result below the 2.5th percentile (i.e. the

mean minus 2 standard deviations) is considered as abnormal .

Neurophysiological Assessment

The EEG was recorded using standardized techniques while the patient, with the eyes closed, laid comfortably in a quiet room. When drowsiness occurred an auditory stimulus was applied by the EEG technician. Five electrodes were attached to the skin at the positions T3, T4, O1, O2 and Cz according to the international "10-20 system" [36]. Electrode impedance was kept lower than 5kΩ. After applying the usual bandpass filters (0.53 - 35 Hz) 2 runs of 100 seconds each were recorded and compared for reproducibility. Artefact free recordings were selected and fed into a computer after AD conversion (sample frequency 102.4 Hz). Ten epochs of 10 seconds each were analyzed by applying Fast Fourier Transformation and the mean power spectrum calculated. Patients are graded in the different stages of hepatic encephalopathy on account of their mean dominant frequency (MDF), and the relative powers of delta and theta activity. In a previous study we have validated this method in 51 healthy controls (median age 41 years, range 21 - 78) and 66 patients with cirrhosis of the liver (median age 60 years, range 21 - 75).[37] A theta activity above 35% (i.e. the mean plus 2 standard deviations in controls) is considered abnormal.

Assessment of daily functioning.

The SIP questionnaire is an often used instrument that assesses the influence of disease and treatment on daily functioning.[24] The questionnaire consists of 136 items, which are grouped into twelve scales: sleep and rest, eating, work, home management, recreation and pastimes, ambulation, mobility, body care and movement (scores of the latter three may be combined as a physical subscore), social interaction, alertness behaviour, emotional behaviour, communication (scores of the latter four may be combined as a psychosocial subscore). Apart from a 12-dimensional profile score and the physical and psychosocial scores, the SIP provides the opportunity to compute a total score. Each score ranges from 0 (best score) to 100 (worst score). Patients mark only items that relate to their health at that time. The SIP has been translated and validated for the Dutch population.[38, 39]

Statistical analysis

Fisher exact test was used to assess differences in sex (male: female), age-group (<60 years: ≥60 years), Child-Pugh score (A:B/C), etiology of liver cirrhosis (alcohol: other) and presence of varices between patients with and without SHE. Difference in median age was tested, using Wilcoxon rank sum test.

Those variables reaching (borderline) statistical significance in the univariate analysis were selected for multivariate analysis, using multiple regression to determine their influence on total SIP score. In this analysis total SIP-scores were logarithmically transformed to reduce skewness of distributions. The limit for statistical significance was set at p=0.05. Test-retest reliability (reproducibility) was assessed using intra-class correlation coefficients. Values above 0.7 generally are considered to indicate good reliability. [40] Internal consistency reliability of the SIP scales was assessed using Cronbach's alpha. [41] The internal consistency of a multi-item scale is a measure of the homogeneity of the items. An alfa of 0.70 is considered to demonstrate good internal consistency. [42]

Results

One hundred and seventy-nine patients with histologically proven cirrhosis (113 male, 66 female, mean age 50 years, SD 14, range 18-77) entered the final analysis. The etiology of cirrhosis was chronic viral hepatitis in 71 patients, alcohol abuse in 38 patients, and other causes (e.g. auto-immune, PBC, cryptogenic) in 70 patients. None of the patients had evidence of neurological and/or psychiatric abnormalities on global clinical examination performed in each patient by one of the first two authors (MG and JCQ). Table 1 summarizes the prevalence of abnormality in age-related psychometric tests and automated EEG analysis found in the study population.

Table 1: Prevalence of abnormal neuropsychological, neurophysiological tests and the diagnosis of SHE in 179 cirrhotic patients without clinical signs of hepatic encephalopathy.

	Number abnormal (%)
Number Connection Test A	15 (9)
Digit Symbol Test	6 (3)
Automated EEG analysis	34 (19)
	Number diagnosed (%)
Subclinical Hepatic Encephalopathy	48 (27)

Forty-eight patients had slowing of the EEG (at least grade 1 by spectral analysis) and/or at least one abnormal psychometric test. These 48 patients were considered as having SHE, while the remaining 131 patients were considered not to have SHE. [15] Clinical and laboratory characteristics of both patient groups are summarized in table 2. The male/female ratio did not differ between groups. Patients with SHE had a more severe liver disease, as quantified by the Child-Pugh score, and more often had esophageal or gastric varices.

Moreover, patients with SHE more often had alcoholic etiology of cirrhosis and they tended to be older: 55 (36-74) years versus 50 (18-77)(median age (range)) years for cirrhotic patients without SHE. However, no difference was found in the number of patients older than sixty years of age (table 2).

Table 2: Clinical and laboratory characteristics of cirrhotic patients with and without SHE.

	SHE + (n = 48)	SHE - (n = 131)	p-value
Male/female ratio	36:12	77:54	0.35
Age (<60:≥60)	33:15	97:34	0.57
Child-Pugh score (A:B/C ratio)*	25:23	111:20	<0.001
Presence of varices (yes:no)*	45:3	56:75	<0.001
Etiology of cirrhosis (alcohol:other)*	17:31	21:110	0.007

*selected for multivariate analysis (table 4)

Figure 1 shows the mean SIP scores of patients with and without SHE, compared to reference scores of the general population.[39] Patients with cirrhosis and SHE reported significantly more impairment in all 12 scales of the SIP, in the psychosocial subscore, the physical subscore, as well as in the total SIP score, compared to cirrhotic patients without SHE (table 3). Separate analysis of the total SIP score in patients with abnormal psychometric tests, or with an abnormal EEG or with both abnormal psychometric test(s) and abnormal EEG showed equally impaired total SIP scores: for psychometric tests 14 (0.4 - 18.6) for EEG: 11.2 (8.4 - 14), and for both psychometric tests and EEG 13.5 (6.9-20.2) versus 4.6 (3.7 - 5.5) (mean (95% CI of mean) for cirrhotic patients with normal test results (no SHE).

The results of the SIP scores were similar between the various etiologies of chronic liver disease. Although patients with SHE more often had cirrhosis of alcoholic etiology, patients with alcoholic cirrhosis did not report more impairments on the psychosocial, physical or on the total SIP score, compared to patients with non-alcoholic cirrhosis (data not shown).

Possible confounders in the analysis comparing patients with and without SHE could be the severity of liver disease (Child-Pugh score, presence of varices) and the etiology of cirrhosis (including fatigue as often observed in viral disease and PBC). Therefore, these variables were selected for multivariate analysis to evaluate their impact on total SIP score. This analysis showed that only presence of SHE and neither the Child-Pugh score, presence of varices nor the etiology of cirrhosis significantly affects total SIP score (table 4).

Reliability testing of the SIP questionnaire in our patient population using internal consistency measures showed that 10 subscales of the SIP fell below the recommended α-coefficient of 0.70 (table 3). This finding suggests, that the items of these SIP scales are not homogeneous in the population of patients with liver cirrhosis; i.e. the items of such a scale do not reliably represent an underlying characteristic. [43] The high internal consistency of the total SIP score is at least partly attributable to the high number of items.
The reproducibility of the SIP questionnaire was high as we found a good correlation between the SIP results in a 3-month interval (table 5). Minor, statistically not significant, differences of mean SIP scores were found between the first and second completed SIP questionnaire, indicating that during the 3 months patients had not systematically changed with regard to the SIP questionnaire.

Table 3: SIP scores of cirrhotic patients with and without SHE.

SIP scales (no. of items)	ref. group	cirrhosis with SHE (n=48)		cirrhosis without SHE (n=131)		p value*	IC
Psychosocial scales							
Social Interactions (20)	4	14.7	(11.1 - 18.2)	7	(5.3 - 8.6)	<0.001	0.7
Alertness (10)	4.7	21.3	(14.3 - 28.2)	8.1	(5.4 - 10.7)	<0.001	0.8
Emotional Behaviour (9)	3.9	10.9	(6.5 - 15.4)	5.0	(3.3 - 6.7)	0.01	0.6
Communication (9)	1.0	5.9	(2.8 - 9)	1.5	(0.4 -2.5)	0.001	0.5
Psychosocial subscore		13.3	(9.7 - 16.9)	5.7	(4.3 - 7.1)	<0.001	0.8
Physical scales							
Ambulation (12)	3.0	7	(4.6-9.3)	3.1	(2.1-4.0)	0.002	0.5
Mobility (10)	2.3	9.4	(5.6-13.1)	2.2	(1.2-3.2)	<0.001	0.6
Bodycare / Movement (23)	1.9	5	(2.9-7.2)	1.2	(0.7-1.6)	<0.001	0.6
Physical subscore		6.3	(4.1 - 8.5)	1.8	(1.2 - 2.4)	<0.001	0.8
Independent scales							
Sleep/Rest (7)	4.7	18.4	(13.5 - 23.4)	8.4	(6.6 - 10.2)	<0.001	0.3
Work (9)	7.3	24.7	(18.3 - 31.2)	8.7	(5.9 - 11.5)	<0.001	0.5
Home management (10)	4.8	14.6	(9.1 - 20)	5.9	(4.5 - 7.3)	0.001	0.5
Recreations / Pastimes (8)	5.8	23.9	(17 - 30.8)	9.8	(7.5 - 12.1)	<0.001	0.6
Eating (9)	1.0	5.1	(3.3 - 7)	2	(1.4 - 2.6)	0.007	0.2
Total SIP score	3.4	12	(9.5 - 14.5)	4.6	(3.7 - 5.5)	<0.001	0.9

Scores are minimally 0 (good performance) and maximally 100 (bad performance). Mean scores are given; 95% CI of mean scores between brackets. Ref. group: reference group 594 dutch citizens (ref. 39), IC: internal consistency (Crohnbach α (pooled data)). *p-value: Wilcoxon rank sum test.

Table 4: Effect of presence of SHE, Child-Pugh score, etiology (alcohol vs. non-alcohol) and presence of varices on total SIP score

variable	regression coefficient	95 % confidence interval	p-value
SHE	3.7	2.0 - 6.7	<0.001
child-pugh	1.2	0.66 - 2.2	0.56
varices	1.4	0.82 - 2.5	0.20
etiology	0.89	0.49 - 1.7	0.74

Table 5: SIP scores of 38 cirrhotic patients at baseline and after 3months

	T = 0 months		T = 3 months		p-value*	intra-class correlation
Psychosocial scales						
Social interactions	10	(6.3 - 13.7)	9.6	(6.2 - 13)	0.82	0.84
Alertness	14	(7.6 - 20.7)	12.6	(5.5 - 19.7)	0.46	0.89
Emotional Behaviour	8.6	(3.4 - 13.8)	6.9	(3.0 - 10.7)	0.77	0.81
Communication	4.6	(1.1 - 8.2)	5.1	(0.9 - 9.2)	0.87	0.82
Psychosocial subscore	9.6	(5.9 - 13.2)	8.8	(5.1 - 12.6)	0.56	0.94
Physical scales						
Ambulation	5.8	(3.1 - 8.4)	5.1	(2.5 - 7.8)	0.69	0.88
Mobility	5.0	(1.5 - 8.5)	5.2	(2.1 - 8.3)	0.85	0.58
Bodycare / movement	2.9	(1.2 - 4.7)	2.9	(1.1 - 4.6)	0.99	0.88
Physical subscore	4.0	(2.1 - 5.9)	3.7	(2.1 - 5.4)	1.0	0.89
Independent scales						
Sleep / rest	11.4	(6.3 - 16.5)	9.3	(5.2 - 13.4)	0.56	0.83
Work	22.9	(15.7 - 30.1)	21.1	(14 - 28.2)	0.67	0.89
Home management	9.2	(5.6 - 12.7)	11.8	(7.2 - 16.5)	0.53	0.67
Recreation / Pastimes	14.7	(8.2 - 21.1)	12	(7.1 - 16.9)	0.88	0.67
Eating	3.6	(2 - 5.2)	2.6	(1.1 - 4.1)	0.25	0.58
Total SIP score	8.5	(5.8 - 11.1)	7.9	(5.3 - 10.5)	0.68	0.96

Mean scores are given; 95% confidence interval of mean between brackets. Statistics: *Wilcoxon rank sum test.

Discussion

We performed a study to determine the influence of SHE on the quality of life. Two psychometric tests with age-related normal values (Number connection test part A and Digit Symbol Test) and automated EEG analysis were selected to be used as neuropsychological and neurophysiological screening tests for SHE.[15] We defined SHE as presence of at least one abnormal psychometric test and/or abnormal slowing of the EEG (at least grade 1 by spectral analysis). Using this definition, we found a SHE prevalence of 27 % in our outpatient cirrhotic population, which is in agreement with the prevalence found in studies using the same methods. [13-15, 17]

We used the SIP as a method for evaluating the influence of SHE on daily functioning in cirrhotic patients, as this questionnaire contains items which resemble the complaints seen in early stages of hepatic encephalopathy. In addition, this questionnaire has been used in a previous study in patients with SHE. [10]

In our study we found a diminished level of functioning in patients with SHE, as reflected by significantly more impairments in all 12 categories of the SIP. Highest scores were found on the categories social interactions, alertness, emotional behaviour, mobility, sleep/rest, home management and recreation and pastimes. These are all items which are expected to be affected in cognitive disorders. Although the severity and etiology of liver disease could have influenced the total SIP scores, multivariate analysis showed that only the presence of SHE independently accounts for the total SIP score result.

Patients with hepatic encephalopathy grade 1 can complain about e.g. sleep disturbances, impaired calculation, shortened attention, mild personality changes and muscular incoordination. [44] Patients with SHE (in literature considered as a preclinical stage of hepatic encephalopathy) should therefore, although to a lesser extent, have similar complaints in daily life. Our findings support this assumption.

How reliable are our findings? First, our study was performed in a large sample size (n=179), which makes the results of the SIP more adequate for statistical analysis. Second, SHE was diagnosed using a combination of neuropsychological and neurophysiological tests. We only used validated psychometric tests with age-related normal values, thereby abolishing the diagnostic bias caused by aging on neuropsychological performance.[15, 17] The fact that patients with abnormal psychometric tests as well as patients with an abnormal EEG showed more impairments in daily life, supports the use of both neuropsychological and neurophysiological methods for the diagnosis of SHE. Third, we used a validated test to evaluate daily functioning, with reference values for the Dutch population. In addition, we found a good test-retest reliability of the SIP in our patient population with liver cirrhosis.

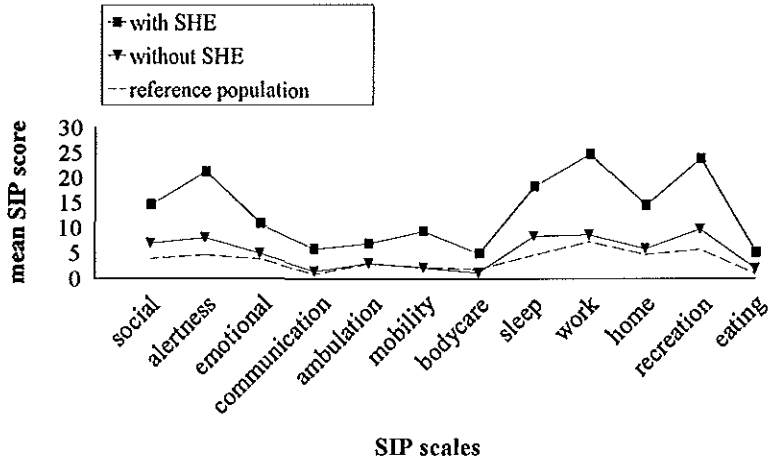

Figure 1 Mean SIP score in 131 cirrhotic patients without SHE and 48 patients with SHE, compared with a reference population of 594 healthy Dutch volunteers (Jacobs et al, ref. 39).

Fourth, we tried to exclude confounding variables by applying multivariate analysis. Age, etiology and severity of liver cirrhosis did not explain the difference in quality of life in cirrhotics with and without SHE; this finding also excludes that symptoms as fatigue (a common symptom in viral disease and in PBC) explain the observed difference.
Lastly, our findings are logical. Should we treat patients with SHE because of the diminished level of daily functioning found in this study? Up to now, no intervention studies have been performed, in which functional status is used as an outcome of treatment efficacy in SHE. Treatment of SHE could be beneficial in patients with stable liver disease and impaired performance at work or daily life. We therefore strongly suggest the use of 'health-related quality of life questionnaires' in trials with treatment specifically directed to SHE.

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References

1. Butterworth RF. Pathogenesis and treatment of Portal-Systemic Encephalopathy: An Update. Dig Dis Sci 1992; 37: 321-7.
2. Parsons-Smith BG, Summerskill WHJ, Dawson AM, Sherlock S. The electroencephalograph in liver disease. Lancet 1957; 2: 867-871.
3. Gitlin N. Subclinical Portal-Systemic Encephalopathy. Am J Gastroenterol 1988; 82: 8-11.
4. Zeegen R, Drinkwater JE, Dawson AM. Method for measuring cerebral dysfunction in patients with liver disease. Br Med J 1970; 2: 633-636.
5. Rikkers L, Jenko P, Rudman D, Freides D. Subclinical hepatic encephalopathy: detection, prevalence, and relationship to nitrogen metabolism. Gastroenterology 1978; 75: 462-469.
6. Gilberstadt SJ, Gilberstadt H, Zieve L, Buegel B, Collier RO, McClain CJ. Psychomotor performance defects in cirrhotic patients without overt encephalopathy. Arch Intern Med 1980; 140: 519-521.
7. Gitlin N, Lewis DC, Hinkley L. The diagnosis and prevalence of subclinical hepatic encephalopathy in apparently healthy ambulant, non-shunted patients with cirrhosis. J Hepatol 1986; 3: 75-82.
8. Sood GK, Sarin SK, Mahaptra J, Broor SL. Comparative efficacy of psychometric tests in detection of subclinical hepatic encephalopathy in nonalcoholic cirrhotics: search for a rational approach. Am J Gastroenterol 1989; 2: 156-9.
9. Schomerus H, Schreiegg J. Prevalence of latent portasystemic encephalopathy in an unselected population of patients with liver cirrhosis in general practice. Z Gastroenterol 1993; 31: 231-234.
10. Tarter RE, Hegedus AM, van Thiel DH, Schade RR, Gavaler JS, Starzl TE. Nonalcoholic cirrhosis associated with neuropsychological dysfunction in the absence of overt evidence of hepatic encephalopathy. Gastroenterology 1984; 86: 1421-1427.
11. Rehnstrom S, Simert G, Hansson J et al. Chronic hepatic encephalopathy. A psychometrical study. Scand J Gastroenterol 1977;12:305-11.
12. Moore JW, Dunk AA, Crawford JR, Deans H, Besson JAO, De Lacey G, Sinclair TS, Mowat NAG, Brunt PW. Neuropsychological deficits and morphological MRI brain scan abnormalities in apparently healthy non-encephalopathic patients with cirrhosis. J Hepatol 1989; 9: 319-325.
13. Koch H, Schauder P, Schäfer G, Dahme B, Ebel W, Vahldiek B, König F, Henning H, Untersuchungen zur diagnose und prävalenz der latenten hepatischen encephalopathie. Z Gastroenterol 1990; 28: 610-615.
14. Weissenborn K, Scholz M, Hinrichs H, Wiltfang J, Schmidt FW, Künkel H. Neurophysiological assessment of early hepatic encephalopathy. Electroenceph Clin Neurophysiol 1990; 75: 289-295.
15. Quero JC, Hartmann IJC, Meulstee J, Hop WCJ, Schalm SW. The diagnosis of subclinical hepatic encephalopathy in patients with cirrhosis using neuropsychological tests and automated electroencephalogram analysis. Hepatology 1996; 24:556-60.
16. Yen CL, Liaw YF. Somatosensory evoked potentials and number connection test in the detection of subclinical hepatic encephalopathy. Hepatogastroenterol 1990; 37: 332-334.
17. Quero JC, Schalm SW. Subclinical hepatic encephalopathy. Seminars in Liver Disease 1996;16:321-8.
18. Schomerus H, Hamster W, Blunck H, Reinhard U, Mayer K, Döll W. Latent portasystemic encephalopathy. I. Nature of cerebral functional defects and their effect on fitness to drive. Dig Dis Sci 1981; 26: 622-630.
19. McClain CJ, Potter TJ, Kromhout JP, Zieve L. The effect of lactulose on psychomotor performance tests in alcoholic cirrhotics without overt hepatic encephalopathy. J Clin Gastroenterol 1984; 6: 325-9.
20. Morgan MY, Alonso M, Stanger LC. Lactitol and lactulose for the treatment of subclinical hepatic encephalopathy in cirrhotic patients. J Hepatol 1989; 8: 208-217.
21. Egberts EH, Schomerus H, Hamster W, Jurgens P. Branched chain amino acids in the treatment of latent

portosystemic encephalopathy. A double-blind placebo-controlled crossover study. Gastroenterology 1985; 88: 887-895.

22. Srivastava A, Mehta R, Rothke SP, Rademaker AW, Blei AT. Fitness to drive in patients with cirrhosis and portal-systemic shunting: a pilot study evaluating driving performance. J Hepatol 1994; 21: 1023-8.
23. Kazis LE. Health outcome assessments in medicine: history, applications and new directions. Adv Intern Med 1991; 36: 109-30.
24. Bergner M, Bobbitt RA, Cartner WB, Gilson BS. The sickness impact profile: development and final revision of a health status measure. Med Care 1981; 19: 787-805.
25. Sullivan M. The Sickness Impact Profile (SIP): an instrument for overall health assessment; a basic evaluation. JDR 1988; 13: 167-9.
26. Tarter RE, Switala J, Arria A, Van Thiel DH. Impact of liver disease on daily living in transplantation candidates. J Clin Epidemiol 1991; 44: 1079-83.
27. Tarter RE, Switala J, Arria A, Plail J, Van Thiel DH. Quality of life before and after orthotopic hepatic transplantation. Arch Intern Med 1991; 151: 1521-6.
28. Tarter RE, Switala J, Plail J, Havrilla J, Van Thiel DH. Severity of hepatic encephalopathy before liver transplantation is associated with quality of life after transplantation. Arch Intern Med 1992; 152: 2097-101.
29. Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC, Perrillo RP, Carey W, Jacobson IM, Payne J, Dienstag JL, Van Thiel DH, Tamburro C, Martino FP, Sanghvi B, Albrecht JK. Assessing health-related quality of life in chronic hepatitis C using the Sickness Impact Profile. Clinical Therapeutics 1994; 16: 334-43.
30. Van der Rijt CCD, Mulder P, Krenning EP, Schalm SW. Encephalopathy in liver disease: potential pathogenetic factors identified by logistic regression analysis. Submitted for publication.
31. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60: 646-649.
32. Reitan RM. Validity of the trail making test as an indication of organic brain damage. Percept Mot Skills 1958; 8: 271-276.
33. Schönleber R. Standardisierung eines psychometrischen Tests zur Erfassung der hepatischen encephalopathie. Thesis. Tübingen 1989.
34. Lezak MD. A compendium of tests and assessment techniques. In: Lezak MD. Neuropsychological assessment. Oxford university press, 1983
35. Stinissen J, Willems PJ, Coetsier P, Hulsman WLL. Handleiding bij de Nederlandstalige bewerking van de Wechsler Adult Intelligence Scale. Swets & Zeitlinger B.V., Lisse, The Netherlands 1970. ISBN 90 265 0208087.
36. Jasper HH. The ten twenty electrode system of the international federation. Electroenceph Clin Neurophysiol 1958; 10: 371-375.
37. Van Der Rijt CCD, Schalm SW, De Groot GH, De Vlieger M. Objective measurement of hepatic encephalopathy by means of automated EEG analysis. Electroenceph Clin Neurophysiol 1984; 57: 423-6.
38. De Melker RA, Touw-Otten F, Jacobs HM, Luttik A. De waarde van de 'Sickness Impact Profile' als uitkomstmeting. Ned Tijdschr Geneeskd 1990; 134: 946-8.
39. Jacobs HM, Luttik A, Touw-Otten FWMM, De Melker RA. De 'Sickness Impact Profile': resultaten van een valideringsonderzoek van de Nederlandse versie. Ned Tijdschr Geneeskd 1990; 134: 1950-2.
40. Streiner DL, Norman GR. Health measurement scales. Oxford: Oxford Medical Publications, 1989 (ISBN 0 19 2617737)
41. Bravo G, Potvin L. Estimating the reliability of continuous measures with cronbach's alpha and intraclass

correlation coefficient: toward the integration of two traditions. J Clin Epidemiol 1991; 44: 381-90.
42. Nunnaly JC. Psychometric theory. McGraw Hill, New York 1978.
43. Essink-Bot ML, Krabbe PFM, Agt HME van, Bonsel GJ. NHP or SIP? A comparative study in renal insufficiency associated anemia. Quality of Life Research 1996; 5: 91-100.
44. Conn HO. The hepatic encephalopathies. In: Conn HO, Bircher J, eds. Hepatic encephalopathy: syndromes and therapies. Bloomington.Medi-Ed press,1994:1-12.

Bedside diagnosis of subclinical hepatic encephalopathy.

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Abstract

Subclinical hepatic encephalopathy adversely affects daily functioning. To determine which elements of daily life have predictive value for subclinical hepatic encephalopathy, a study was performed in 179 outpatients with liver cirrhosis. Subclinical hepatic encephalopathy was diagnosed using psychometric tests with normal values corrected for age (Number Connection Test A and the Digit Symbol Test) and automated analysis of the electroencephalogram (EEG). Daily functioning was measured with the Sickness Impact Profile (SIP), a quality of life questionnaire, containing 136 statements. Patients with and without SHE were compared for differences in response to all statements by univariate analysis, and subsequently by multivariate analysis of potential discriminating statements. SHE was diagnosed in 48 patients (27%). Thirty-six statements were significantly more often true for patients with subclinical hepatic encephalopathy. Multivariate analysis showed, that five statements of the SIP related to alertness, sleep and rest, fine motor skills and work, have independent predictive power for subclinical hepatic encephalopathy. Combining these statements predictive for subclinical hepatic encephalopathy with patient characteristics enables physicians to assess the probability of subclinical hepatic encephalopathy in the individual cirrhotic patient.

Introduction

The clinical syndrome of hepatic encephalopathy (HE) is characterized by disturbance of mental state and neuromuscular function. In clinical practice, hepatic encephalopathy is classified in four grades; with progressively more advanced deterioration of mental state with each grade.[1] In addition to clinically manifest HE, a subclinical stage has been described. [2-4] The prevalence of subclinical hepatic encephalopathy (SHE) is estimated to vary from 14% in patients with Child-Pugh A cirrhosis to 45% in patients with Child-Pugh B/C cirrhosis, according to recent studies using appropriate methods [3-5]. The clinical relevance of SHE is still subject to debate. Evidence is increasing that SHE adversely affects daily functioning. [6,7]

In the recent study, the effect of SHE on daily functioning was tested, using the Sickness Impact Profile (SIP). [7] The SIP is a generic, non disease-specific quality of life questionnaire. [8]

We hypothesized that the SIP questionnaire contains a number of statements on daily functioning, that could be specifically abnormal in SHE. Identification of a subgroup of cirrhotic patients with a high probability of SHE by simple means would be clinically relevant, since confirmatory testing by neuropsychological and neurophysiological methods can then be limited considerably. In the present study we analysed the value of selected SIP statements for the diagnosis of SHE.

Patients and methods

Patients

From January 1, 1992 to May 1, 1996 202 consecutive patients attending the outpatient clinic of Hepatogastroenterology of the University Hospital Rotterdam were screened for SHE. Inclusion criteria were: histologically proven cirrhosis of the liver and age between 16 and 80 years. 193 of 202 patients fitted the inclusion criteria; 3 patients had a surgically constructed portacaval shunt (no cirrhosis) and 6 patients had only clinical signs of cirrhosis at screening. Exclusion criteria were: clinical manifest hepatic encephalopathy *, history of recent (less than 6 weeks) alcohol abuse, use of benzodiazepines, anti-epileptics, or other psychotropic drugs, inability to perform the psychometric tests and complete the SIP questionnaire (due to insufficient knowledge of the Dutch language or bad vision), and severe medical problems such as congestive heart failure, pulmonary disease, neurologic disease etc. that influence the quality of life measurement. In the final analysis, a total of 179 patients with histologically proven cirrhosis of the liver were included. 14 patients were excluded because of incomplete SHE screening and/or inability to fill out the SIP questionnaire.

All patients underwent a clinical and laboratory investigation, psychometric tests, and automated EEG analysis. In addition, the SIP questionnaire was administered.

Methods

Diagnosis of SHE. SHE was defined as the presence of at least one abnormal psychometric test and /or abnormal slowing of the electroencephalogram (EEG). [4]

Number Connection Test part A (NCT-A) The NCT-A is a derivative from the Trail Making Test [11] and measures cognitive motor abilities. Patients perform the test by connecting numbers printed on paper consecutively from 1 to 25. Age-related normal values of the NCT-A have been developed in 681 persons without liver disease. [12] A test is considered abnormal, if the time needed to perform the test is above the 97.5th percentile of the age-group.

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*Note: Clinically manifest hepatic encephalopathy was assumed to be present if at least 2 of the following abnormalities: inverted sleep pattern, impaired calculation, disturbed secondary memory, and slowness of speech were detected, or one abnormality in association with a flapping tremor.[9] Impaired calculation was assessed by asking the patient to subtract serial sevens from 100 and to repeat a series of six nonconsecutive numbers in the same order (a normal individual can easily remember seven numbers forwards). Memory was tested by asking the patient to remember three objects and to repeat these several minutes later, and by asking the patient about past prime ministers, dates of wars, and events that affect everyone.

Digit Symbol Test (DST) This is a subtest of the Wechsler Adult Intelligence Scale (WAIS) and measures motor speed and accuracy. [13] The patient is given a list of digits associated with symbols from 1 to 9 and is asked to fill in blanks with symbols that correspond to each number. Age-related normal values have been developed in 2169 Dutch and Belgian control subjects without liver disease. [14] A total number of symbols below the 2.5th percentile of the age-group is considered as abnormal.

Spectral analysis of the EEG. The EEG was recorded using standardized techniques. Five electrodes were attached to the skin at the positions T3, T4, O1, O2 and Cz according to the international "10-20 system". Electrode impedance was kept lower than 5kΩ. After applying the usual bandpass filters (0.53 - 35 Hz) 2 runs of 100 seconds each were recorded and compared for reproducibility. Artefact free recordings were selected and fed into a computer after AD conversion (sample frequency 102.4 Hz). Ten epochs of 10 seconds each were analysed by applying Fast Fourier Transformation and the mean power spectrum was calculated. Patients are graded in the different stages of hepatic encephalopathy on account of their mean dominant frequency (MDF), and the relative powers of delta and theta activity. [15] A theta activity above 35% (i.e. the mean plus 2 standard deviations in controls) is considered abnormal.

Assessment of daily functioning. The Sickness Impact Profile (SIP) was used to assess daily functioning. The questionnaire consists of 136 statements, which are grouped into twelve scales: sleep and rest, eating, work, home management, recreation and pastimes, ambulation, mobility, body care and movement, social interaction, alertness behaviour, emotional behaviour and communication. [12] Each score ranges from 0 (best score) to 100 (worst score). Patients are instructed to mark only items that relate to their health at that time. It takes a patient about 20 minutes to complete the SIP. The SIP questionnaire has been validated for the Dutch population [16,17].

Liver function assessment. The Child-Pugh score was used to assess the severity of liver disease. [10] Three biochemical variables (serum albumin, bilirubin and prothrombin time) in addition to two clinical characteristics (presence or absence of ascites and clinical signs of hepatic encephalopathy) determine the Child-Pugh score. Each variable is given one to three points, leading to scores ranging from 5 (excellent liver function) to 15 points (poor liver function). Presence of esophageal or gastric varices was evaluated using reports of endoscopic investigations in 174 patients, and esophageal X-rays in 5 patients. Alcoholic etiology of liver cirrhosis was defined as: presence of severe alcohol abuse in patient history, defined as at least 6 drinks a day for several years.

Statistical analysis. Fisher exact test was used to assess differences in sex (male:female), age-group (age under 60 years versus older than 60 years), Child-Pugh group (A:B/C), etiology of liver cirrhosis (alcohol: other) and presence of esophageal varices between patients with and without SHE. The differences of each of the 136 statements of the SIP separately was tested, using the Fisher exact test. Statements which differed between groups in univariate comparisons ($p<0.05$) entered into multivariate analysis (backward elimination logistic regression, using the package LOGXACT), together with the patient characteristics: age-group, sex, Child-Pugh score, etiology and presence of esophageal varices. Regression coefficients of those variables with predictive power for SHE ($p<0.05$) were used to construct a SHE score.
The true positive rate (sensitivity) and the false positive rate (1 - specificity) of the total SIP score, the score of the statistically significant statements, and of the SHE score were evaluated, by calculating the receiver operating characteristic (ROC). A ROC curve shows the relationship between the true positive rate and false positive rate of a diagnostic test. Every point on the curve represents the characteristics of a certain "cut-off point" of the test. A test without discriminatory capacity for two groups would give a line of 45 degrees. The greater the discriminatory capacity of the test, the more the curve will approach the point which represents 100% true positive rate and 0% false positive rate. A test without discriminatory capacity has an area under the curve (AUC) of 0.50; a perfect test would have an AUC of 1.0. In clinical practice diagnostic tests usually have an AUC ranging from 0.60 to 0.90. [18]

Results

One hundred and seventy-nine patients with histologically proven cirrhosis (113 male, 66 female, mean age 50 years, SD 14, range 18-77) entered the study. The etiology of cirrhosis was chronic viral hepatitis in 71 patients, past alcohol abuse in 38 patients, and other causes (e.g. auto-immune, PBC, cryptogenic) in 70 patients. Forty-eight out of 179 patients were diagnosed as having SHE (27%). Twenty-one patients (12%) either had one or two abnormal psychometric tests and 34 patients (19%) had abnormal slowing of the EEG. Ten patients (6%) had an abnormal psychometric test as well as abnormal slowing of the EEG.
No difference in sex ratio, nor in age group (age under 60 years of age versus 60 years and older) between cirrhotic patients with and without SHE was found (data not shown). Cirrhotic patients with SHE had a more severe liver disease (percentage of patients with Child-Pugh B/C: 48 % vs. 15 % ($p<0.001$); percentage of patients with esophageal varices: 94 % vs. 43 % ($p<0.001$); in addition, more patients with SHE had an alcoholic etiology of liver disease (35 % vs. 16 % ($p=0.007$)). Univariate analysis of each of the SIP statements showed, that thirty-six out of 136 SIP statements were significantly more frequent in patients with SHE (table 1).

Table 1 SIP statements significantly different for SHE positive and negative patients selected for multivariate analysis

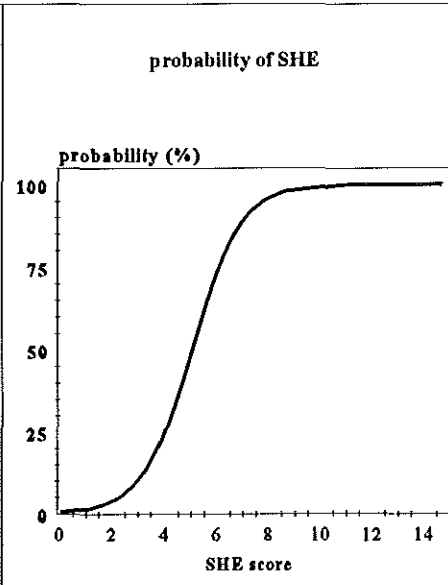
Category	Statement		SHE + (n=48)	SHE - (n=131)
ambulation	1.	I walk more slowly.	46	24
	2.	I walk shorter distances or stop to rest often.	23	4
	3.	I walk by myself but with some difficulty, for example, limp, wobble, stumble, have stiff leg.	13	1
mobility	4.	I am staying in bed more.	13	2
	5.	I am staying in bed most of the time.	6	0
	6.	I stay away from home only for brief periods of time.	31	5
bodycare/ movement	7.	I do not fasten my clothing, for example, require assistance with buttons, zippers, shoelaces.	6	0
	8.	I stand only for short periods of time.	33	7
	9.	I change position frequently.	19	8
	10.	I do not have control of my bowels.	8	1
	11.	I have trouble getting shoes, socks or stockings on.	13	1
social interactions	12.	My sexual activity is decreased.	58	27
	13.	I am going out less to visit people.	42	22
	14.	I am cutting down the length of visits with friends.	29	14
	15.	I often express concern over what might be happening to my health.	27	13
	16.	I stay alone much of the time.	25	9
	17.	I show less interest in other people's problems, for example, don't listen when they tell me about their problems, don't offer to help.	17	4
alertness	**18.**	**I am confused and start several actions at a time.**	23	3
	19.	I react slowly to things that are said or done.	25	5
	20.	I sometimes behave as if I were confused or disorientated in place or time, for example, where I am, who is around, directions, what day it is.	13	2
	21.	**I forget a lot, for example, things that happened recently, where I put things, appointments.**	46	15
	22.	I do not keep my attention on any activity for long.	23	11
emotional behaviour	23.	I act irritable and impatient with myself, for example, talk badly about myself, swear at myself, blame myself for things that happen.	23	9
	24.	I often moan and groan in pain or discomfort.	15	4
	25.	I keep rubbing or holding areas of my body that hurt or are uncomfortable.	27	5
communication	26.	I am having trouble writing or typing.	13	3
sleep and rest	27.	I am sleeping or dozing most of the time - day and night.	10	0
	28.	**I spend much of the day lying down in order to rest.**	15	0
	29.	I lie down more often during the day in order to rest.	40	19

Table 1 (continued)

home management	30.	I am not doing any of the regular work around the house that I would usually do.	10	2
	31.	**I have difficulty doing handwork, for example, turning faucets, using kitchen gadgets, sewing, carpentry.**	13	2
	32.	I am not doing any of the clothes washing that I would usually do.	8	1
recreation/ pastime	33.	I am cutting down on some of my usual inactive recreation and pastimes, for example, watching TV, playing cards, reading	23	7
	34.	I am going out for entertainment less often.	36	17
	35.	I am not doing any of my usual physical recreation or activities.	29	9
work	**36.**	**I am not working at all.**	50	15

In the multivariate analysis, the predictive power of the 36 selected statements over that of the Child-Pugh score, age, sex, etiology of liver disease, and presence of esophageal varices was evaluated. Five statements (table 1: no. 18, 21, 28, 31 and 36) had independent predictive value for SHE. On the basis of this analysis, a formula for a SHE score was constructed. Subsequently, the relation between the probability of SHE and the SHE score was analysed; the result is graphically presented in figure 1, together with four examples. ROC analysis shows, that both the total SIP score (AUC = 0.78) as well as the score of the 36 statements (AUC = 0.81) are able to differentiate patients with and without SHE (figure 2). The ROC curve of the SHE score has highest discriminatory capacity for SHE (ROC=0.91). This underlines, that the predictive value of the SHE score for the diagnosis SHE is markedly improved by combining information from the SIP questionnaire with patient characteristics that reflect the prevalence of the syndrome. A cut-off level of 3.3 for the SHE score leads to a sensitivity of 90%. The false positive rate associated with this cut-off level equals 24%. If the cut-off level is chosen in such a way that a sensitivity of 99% is obtained (SHE score of 1.2), the false positive rate increases considerably (up to 85 %).

Items	factor	SHE score
statement: " I am confused and start several actions at a time "	1.8	
statement: " I forget a lot, e.g. things that happened recently, where I put things, appointments "	1.1	
statement: " I spend much of the day lying down in order to rest "	3.7	
statement: " I have difficulty doing handwork, for example, turning faucets, using kitchen gadgets, sewing, carpentry "	1.9	
statement: "I am not working at all "	1.3	
Male sex	1.2	
Child-Pugh ≥ 7 (B/C)	1.6	
varices present	2.1	
TOTAL SCORE:	→	



Examples		SHE score	p
No statements, male sex, Child-Pugh A, varices present:	→	3.3	14 %
No statements, male sex, Child-Pugh C, varices present:	→	4.9	46 %
Statement 1 and 2 present, male sex, Child-Pugh C, varices present:	→	7.8	95 %
Statements 1-5 present, male sex, Child-Pugh C, varices present:	→	14.7	100 %

Figure 1: Calculation and interpretation of the SHE score. The SHE score is calculated as follows: male cirrhotic patient: 1.2 is added up to score; Child-Pugh B/C: 1.6 is added up to score; presence of esophageal varices: 2.1 is added up to score; presence of either of the 5 statements: factors are added up to score. With the calculated SHE score the probability of SHE can be derived from the figure. Four examples illustrate the applicability of the SHE score.

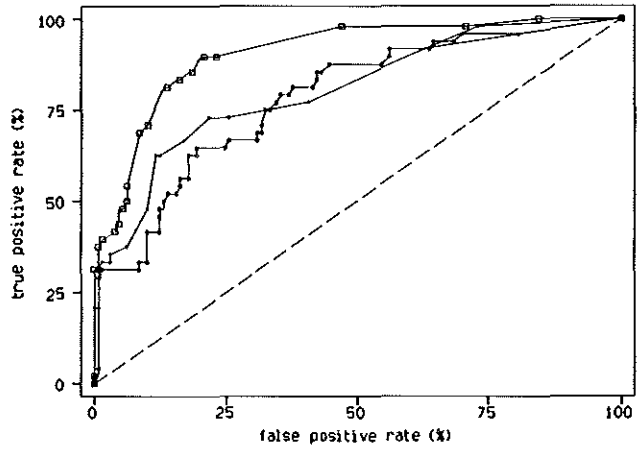


Figure 2: Diagnostic capacity of the SHE score (□), the score of the 36 statistical significant SIP statements (+) and the total SIP score (o) for the diagnosis SHE (ROC curves).

Discussion

Cirrhotic patients with SHE report more impairments on daily functioning using the Sickness Impact Profile questionnaire. Evaluation of the 136 separate statements showed that 36 statements are marked more often by SHE patients. After detailed statistical analysis five statements were selected as predictive for SHE. These five questions in combination with patient characteristics as sex, Child-Pugh score and presence of esophageal varices will allow physicians to estimate the probability of SHE and subsequently order confirmatory tests.

Setting the cut-off level of the SHE score at 3.3 (90% sensitivity) implies an acceptable percentage of false positives (24 %). At a sensitivity of 99% the false positive rate increases considerable (up to 71 %), which is not acceptable for an initial screening test. [18]

In this study SHE was diagnosed by using a combination of neuropsychological and neurophysiological tests. Internationally, there is an ongoing debate over how to diagnose SHE. We used well validated methods with normal values corrected for age, that yielded a low

percentage of SHE positives in Child-Pugh A cirrhotics, and appreciable numbers in Child-Pugh B/C cirrhotics. [4] We think that this combination of tests is clinically appropriate for the diagnosis of SHE, although in the future some adaptations may be needed in view of international consensus. [4,5]

Symptoms of early hepatic encephalopathy - a term which might replace the term subclinical hepatic encephalopathy - include: memory (impaired computation), affect (euphoria or depression), attentiveness, language, judgement and cognitive function, inversion of sleep pattern, and muscular incoordination (impaired handwriting). [2-4] The list of 36 statements that were the basis for the multivariate analysis indicates, that most impairments are found in the categories: alertness (forgetfulness, confusion, attentiveness), sleep and rest (sleeping, dozing during the day), home management (handwork) and communication (writing and typing) (table 1). The statement: " I do not work at all " also had predictive power for SHE; 50% of cirrhotic patients with SHE did not have regular employment, compared to 15% of patients without SHE.

The five statements with predictive value are most useful when assessed in the light of additional risk factors: severity of liver disease (Child-Pugh score) and portal hypertension (esophageal varices). This reflects the variation in predictive value of the test in relation to the prevalence of the syndrome. The relation between SHE and the severity of liver disease has been described previously. [4,19,20] In addition,the presence of portal-systemic shunting is a well-known risk factor, even in the absence of cirrhosis. [21]

This study suggests, that the probability of SHE can be estimated simply at the bedside or in the outpatient clinic. Only cirrhotic patients with a high probability for SHE should undergo electrophysiologic and psychometric tests to confirm the diagnosis. The SHE score can be used for the diagnosis of SHE, but might also be of value in intervention studies for SHE.

References

1. Conn HO. The hepatic encephalopathies. In: Conn HO, Bircher J, editors. Hepatic encephalopathy: syndromes and therapies. Bloomington: Medi-Ed press; 1994. p. 1-12.
2. Gitlin N, Lewis DC, Hinkley L. The diagnosis and prevalence of subclinical hepatic encephalopathy in apparently healthy ambulant, non-shunted patients with cirrhosis. J Hepatol 1986;3:75-82.
3 Weissenborn K, Scholz M, Hinrichs H, Wiltfang J, Schmidt FW, Künkel H. Neurophysiological assessment of early hepatic encephalopathy. Electroenceph Clin Neurophysiol 1990;75:289-95.
4. Quero JC, Hartmann IJC, Meulstee J, Hop WCJ, Schalm SW. The diagnosis of subclinical hepatic encephalopathy in patients with cirrhosis using neuropsychological tests and automated electroencephalogram analysis. Hepatology 1996;24:556-60.
5. Quero JC, Schalm SW. Subclinical hepatic encephalopathy. Semin Liver Dis 1996;16:321-8.
6. Schomerus H, Hamster W, Blunck H, Reinhard U, Mayer K, Dölle W. Latent portosystemic encephalopathy. Nature of cerebral function defects and their effect on fitness to drive. Dig Dis Sc 1981;26:622-30.
7. Groeneweg M, Quero JC, Essink-Bot ML, Hop WCJ, Schalm SW. Subclinical hepatic encephalopathy impairs daily functioning. Hepatology 1998;28:45-49.

8. Bergner M, Bobbitt RA, Cartner WB, Gilson BS. The sickness impact profile: development and final revision of a health status measure. Med Care 1981;19:787-805.
9. Van der Rijt CCD, Mulder P, Krenning EP, Schalm SW. Encephalopathy in liver disease: potential pathogenetic factors identified by logistic regression analysis. Submitted for publication.
10. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646-9.
11. Reitan RM. Validity of the trail making test as an indication of organic brain damage. Percept Mot Skills 1958;8:271-6.
12. Schönleber R. Standardisierung eines psychometrischen Tests zur Erfassung der hepatischen encephalopathie [dissertation]. Tübingen (FRG);1989.
13. Lezak MD. A compendium of tests and assessment techniques. Neuropsychological assessment. Oxford University Press, 1983.
14. Stinissen J, Willems PJ, Coetsier P, Hulsman WLL. Handleiding bij de Nederlandstalige bewerking van de Wechsler Adult Intelligence Scale. Swets & Zeitlinger B.V., Lisse, The Netherlands 1970. ISBN 90 265 0208087.
15. Van der Rijt CCD, Schalm SW, De Groot GH, De Vlieger M. Objective measurement of hepatic encephalopathy by means of automated EEG analysis. Electroenceph Clin Neurophysiol 1984;57:423-6.
16. De Melker RA, Touw-Otten F, Jacobs HM, Luttik A. De waarde van de 'Sickness Impact Profile' als uitkomstmeting. Ned Tijdschr Geneeskd 1990;134:946-8.
17. Jacobs HM, Luttik A, Touw-Otten FWMM, De Melker RA. De 'Sickness Impact Profile': resultaten van een valideringsonderzoek van de Nederlandse versie. Ned Tijdschr Geneeskd 1990;134:1950-2.
18. The selection of diagnostic data. In: Sackett DL, Haynes RB, Guyatt GH, Tugwell R, editors. Clinical epidemiology. A basic science for clinical medicine. 2nd ed. Boston: Little, Brown & Comp.; 1991. p. 69-152.
19. Tarter RE, Sandford SL, Hays AL, Carra JP. Hepatic injury correlates with neuropsychologic impairment. Intern J Neuroscience 1989;44:75-82.
20. Gilberstadt SJ, Gilberstadt H, Zieve L, Buegel B, Collier RO, McClain CJ. Psychomotor performance defects in cirrhotic patients without overt encephalopathy. Arch Intern Med 1980;140:519-21.
21. Sarin SK, Nundy S. Subclinical encephalopathy after portosystemic shunts in patients with non-cirrhotic portal fibrosis. Liver 1985;5:142-6.

The prognostic significance of subclinical hepatic encephalopathy.

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Abstract

Subclinical hepatic encephalopathy may have prognostic significance with regard to the development of clinical hepatic encephalopathy and survival. We studied 116 consecutive patients with histologically proven cirrhosis of the liver for subclinical hepatic encephalopathy, using Number Connection Test A, Digit Symbol Test and spectral analysis of the electroencephalogram. Twenty-five patients (22%) were diagnosed as having subclinical hepatic encephalopathy. Patients with subclinical hepatic encephalopathy were older, had a higher Child-Pugh score, more often had esophageal and/or gastric varices and episode(s) of clinical hepatic encephalopathy in history. During a median follow-up of 29 months (range 1-49 months) patients with subclinical hepatic encephalopathy significantly more often had episodes of clinical hepatic encephalopathy. Survival, however, was similar to patients without subclinical hepatic encephalopathy, and was mainly determined by the Child-Pugh score. The Child-Pugh score was also superior to subclinical hepatic encephalopathy in predicting episodes of clinical hepatic encephalopathy. Therefore the prognostic significance of subclinical hepatic encephalopathy appears limited.

Introduction

The spectrum of hepatic encephalopathy traditionally includes four stages.[1,2] More than twenty years ago, the presence of mental impairment preceding clinical hepatic encephalopathy has been suggested.[3] This preclinical stage, in which patients with cirrhosis demonstrate neuropsychological or neurophysiological deficits, without altered mental state or neurological abnormalities on global clinical examination, was later defined as subclinical hepatic encephalopathy (SHE).[4-7] The prevalence of SHE varies between 30 - 84% in patients with liver cirrhosis, dependent on the diagnostic criteria used.[8-10] A combination of neuropsychological and neurophysiological parameters appears most appropriate for the diagnosis of SHE,[5-8] since both methods probably assess different brain functions. [10] The significance of the diagnosis SHE is subject to debate.[8,10,11] Recent studies show, that SHE has a negative influence on daily functioning.[11,12] In addition, several investigators suggest, that there is a relation between SHE and the subsequent development of episodes of clinical hepatic encephalopathy. [4,7,13] However, studies on the natural history of SHE and its prognosis with respect to development of clinical hepatic encephalopathy and survival are lacking. [4,10] In the present study we therefore focussed on the natural history and prognostic significance of SHE with respect to development of clinical hepatic encephalopathy and survival.

Patients and methods

Patients

From January 1, 1992 to December 31, 1993, 117 consecutive patients with histologically proven cirrhosis visiting the outpatient clinic of the department of Hepatogastroenterology in our hospital, were screened for SHE. Patients with clinical signs of hepatic encephalopathy (impaired intellectual function, altered level of consciousness, personality disorders and/or neuromuscular dysfunction (asterixis)), as well as patients with signs of neurological and/or psychiatric abnormalities on global clinical examination were excluded. Patients with a history of recent excessive alcohol abuse, patients using psychotropic drugs (e.g. benzodiazepines, anti-epileptics) were also excluded. The closing date of follow-up was the first outpatient visit following January 1, 1996. If death occurred before the closing date, the time between screening and date of death was considered as the follow-up period. If routine check-up at the outpatient department was discontinued, or if the next appointment was made in the second half of 1996, the patient's general practitioner was contacted to obtain the missing data.

The median period of observation was 29 months (range 1 - 49 months). One hundred and seventeen patients entered the study. One of the 117 patients was lost of follow-up shortly after screening for SHE, and therefore did not enter the study. The patient characteristics at entry of the 116 remaining subjects are listed in table 1. Liver transplantation (15 patients), shunt-operations (6 patients) and/or TIPS (7 patients) were regarded as an event, indirectly expressing the severity of the liver disease, and therefore patients undergoing these interventions were not excluded from the analysis.

The etiology of liver disease was divided in chronic viral hepatitis, alcohol abuse, and other causes (e.g: auto-immune hepatitis, primary biliary cirrhosis). At entry, the severity of liver disease was assessed in all patients, using the Child-Pugh score. At entry, in 107 patients presence (85 patients) or absence (22 patients) of oesophageal varices was documented. Patients were considered as having decompensated cirrhosis at entry if one of the following complications were present in the patient history: variceal bleeding, jaundice, ascites and clinical hepatic encephalopathy. Variceal bleeding was diagnosed, using the following criteria: signs of haematemesis and/or melena, gastric aspirate containing blood, and the following findings at endoscopy: active bleeding varix or endoscopic signs of recent variceal bleeding, without evidence for other sources of gastro-intestinal bleeding. [14] Ascites was defined as present, if there was ascites on clinical examination (shifting dullness), and/or on abdominal ultrasonography.

Methods

Diagnosis of SHE. SHE was defined as presence of at least one abnormal psychometric test and/ or abnormal slowing of the electroencephalogram (EEG). [8]

Number Connection Test part A (NCT-A) The NCT-A is a derivative from the Trail Making Test [16] and measures cognitive motor abilities. Patients perform the test, by connecting numbers printed on paper consecutively from 1 to 25. Age-related normal values of the NCT-A have been developed in 681 persons without liver disease. [17] A test is considered abnormal, if the time needed to perform the test is above the 97.5th percentile of the age-group.

Digit Symbol Test (DST) This is a subtest of the Wechsler Adult Intelligence Scale (WAIS) and measures motor speed and accuracy. [10] The patient is given a list of digits associated with symbols from 1 to 9 and is asked to fill in blanks with symbols that correspond to each number. Age-related normal values have been developed in 2169 Dutch and Belgian control subjects without liver disease. [14] A total number of symbols below the 2.5th percentile of the age-group is considered as abnormal.

Spectral analysis of the EEG. The EEG was recorded using standardized techniques. Five electrodes were attached to the skin at the positions T3, T4, O1, O2 and Cz according to the international "10-20 system". Electrode impedance was kept lower than 5kΩ. After applying the usual bandpass filters (0.53 - 35 Hz) 2 runs of 100 seconds each were recorded and compared for reproducibility. Artefact free recordings were selected and fed into a computer after AD conversion (sample frequency 102.4 Hz). Ten epochs of 10 seconds each were analysed by applying Fast Fourier Transformation and the mean power spectrum was calculated. Patients are graded in the different stages of hepatic encephalopathy on account of their mean dominant frequency (MDF), and the relative powers of delta and theta activity. [15] A theta activity above 35% (i.e. the mean plus 2 standard deviations in controls) is considered abnormal.

Assessment of liver function. The Child-Pugh score was determined to assess the severity of cirrhosis, including three biochemical variables (serum albumin, bilirubin, and prothrombin time) and two clinical characteristics (presence or absence of ascites and clinical hepatic encephalopathy). [19] The Child-Pugh score ranges from 5 points (excellent liver function) to 15 points (poor liver function). A patient has a Child-Pugh A cirrhosis, if the score is ≤ 6 points; Child-Pugh B if it is 7 - 9 points and Child-Pugh C if the score is > 9 points. Patients without signs of ascites, but on diuretic treatment because of previous ascites scored 2 points for ascites in the Child-Pugh score. In the final analysis patients with Child-Pugh B and C were pooled, as the number of Child-Pugh C patients was too low to justify separate grouping.

Outcome measure. Survival status and clinical hepatic encephalopathy were assessed at the end of follow-up. Of all patients who died during follow-up, the cause of death was documented and defined as liver related death if death was the direct consequence of the patient's underlying liver disease. All other causes of death were considered as non-liver disease related. Episodes of clinical hepatic encephalopathy were classified as precipitated, if the episode occurred in the course of e.g. gastro-intestinal bleeding, constipation, infection or psychotropic medication. If no other cause than the underlying cirrhosis could be found, the episode of clinical hepatic encephalopathy was considered endogenous.

Statistical analysis. Differences in clinical and laboratory characteristics between groups were tested with the Wilcoxon rank sum test or Fisher Exact test. The incidence of clinical hepatic encephalopathy as well as other decompensating events in patients was analysed using the Kaplan-Meier method. In the survival analysis only liver-related deaths were counted. The effect of patient characteristics at screening (presence of SHE, Child Pugh score, age, and clinical hepatic encephalopathy in history) was tested using logrank tests. Those variables with borderline significance (p=0.05) were selected for Cox regression analysis, to estimate the independent prognostic significance of each of the selected variables.

Results

At screening, 25 of 116 (22%) patients were diagnosed as having SHE. Sixteen patients had an abnormal spectral EEG, and nine patients had one or two abnormal psychometric test results; 3 patients had both an abnormal spectral EEG and abnormal psychometric test(s). Patients with SHE were older and had a more severe liver cirrhosis at entry, as quantified by the Child-Pugh score. In addition, esophageal and gastric varices were more often seen in patients with SHE. Nineteen patients (76%) with SHE had a past history of decompensated cirrhosis at entry, compared to 52 patients (57%) without SHE. Patients with SHE more often had episodes of clinical hepatic encephalopathy in history. Other signs of decompensation in history (jaundice or ascites) were equally distributed in both groups (table 1).

Table 1 Patient characteristics at entry (n=116)

	Total group	SHE +	SHE -	p-
Age (mean±SD(range))	49 ± 14 (17-77)	59 ± 11 (36-77)	46 ± 14.0 (17--73)	0.006
Sex (m:f)	85:31	19:6	66:25	0.8
Etiology (N (%))				
viral	58 (50)	11 (44)	47 (52)	0.84
alcohol	22 (19)	8 (32)	14 (15)	0.17
other	36 (31)	6 (24)	30 (33)	0.64
Child-Pugh (N (%))				
A	81 (70)	11 (44)	70 (77)	
B/C	35 (30)	14 (56)	21 (23)	0.003
Varices (N (%))	85 (79)	23 (92)	62 (67)	0.02
Interval between histological diagnosis and entry (median (range))	51 (0-258)	29 (0-184)	57 (0-258)	0.29
Past history of decompensated cirrhosis (N (%))	71 (61)	19 (76)	52 (57)	0.11
encephalopathy	11 (9)	7 (28)	4 (4)	0.002
variceal bleeding	32 (28)	10 (40)	22 (24)	0.13
jaundice	40 (34)	6 (24)	34 (37)	0.24
ascites	43 (37)	13 (52)	30 (33)	0.1

Ten patients with SHE at screening showed signs of clinical hepatic encephalopathy during follow-up, compared to 6 patients without SHE (actuarial percentage at three years: 56% and 8% respectively; $p<0.001$), (figure 1, table 2). Separate analysis of precipitated episodes and endogenous episodes of clinical hepatic encephalopathy showed, that precipitated episodes were seen in 4 patients with SHE and in 2 patients without SHE (actuarial percentage at three years: 13% and 3% respectively; $p=0.008$).

Non-precipitated episodes of clinical hepatic encephalopathy were seen in 6 patients with SHE, versus 4 patients without SHE (actuarial percentage at three years: 32% and 5% respectively; $p=0.006$). Five out of 11 patients with episodes of clinical hepatic encephalopathy in history had clinical hepatic encephalopathy during follow-up, compared with 11 out of 105 patients without clinical hepatic encephalopathy in history (actuarial percentage at three years: 46% versus 21%; $p=0.01$).

Table 2 Mean follow-up, number of decompensating events after follow-up, liver related death and total death.

	SHE + (N=25)	SHE - (N=91)	p-value
Follow-up (mean (range))	25.6 (2-39)	28.8 (1-49)	0.21
Decompensating events in follow-up (%)			
encephalopathy	10 (40)	6 (7)	<0.001
variceal-bleeding	3 (12)	10 (11)	1.0
jaundice	2 (8)	8 (9)	1.0
ascites	5 (25)	15 (16)	0.09
Liver related death	4 (16)	5 (6)	0.43
Total death	6 (24)	12 (13)	0.22

Fourteen out of 35 patients with Child-Pugh score B/C cirrhosis and 2 patients with Child-Pugh A cirrhosis developed clinical hepatic encephalopathy during follow-up (actuarial percentage at three years: 58% and 4% respectively; p<0.001). In Child-Pugh B/C patients a trend was found to more clinical hepatic encephalopathy in patients with SHE (actuarial percentage at three years: 78% versus 38%; p=0.12). For patients with Child-Pugh A cirrhosis, such a trend could not be found. Although patients with SHE were older than patients without SHE, no relation between the development of clinical hepatic encephalopathy and age could be found (data not shown).

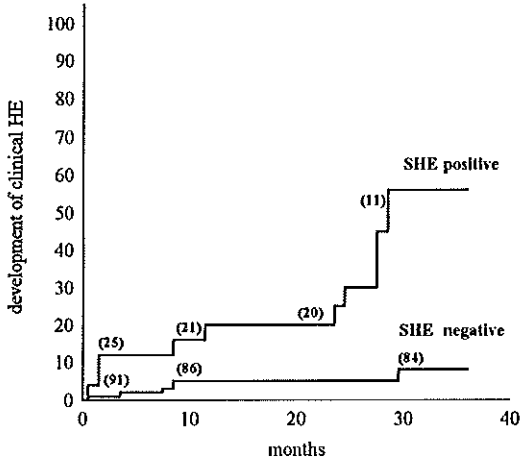


Figure 1 Probability of clinical hepatic encephalopathy in patients with SHE (p<0.001: logrank test)

Evaluating the factors SHE, Child-Pugh score and clinical hepatic encephalopathy in history with regression analysis, we found that both SHE and Child-Pugh score are independent predictors for the development of clinical hepatic encephalopathy (table 3).

During the period of observation eighteen patients died. In 9 cases death was due to progressive liver failure (n=1), hepatocellular carcinoma (n=3) and acute gastro-intestinal bleeding (n=5). In 9 patients death was related to extrahepatic causes: malignancies (n=3), fatal bleeding from duodenal ulcer (n=1), cardiac decompensation (n=3), and suicide (n=1). No differences in death rates during follow-up were seen between SHE positive and SHE negative patients (table 2). Figure 2 shows the actuarial survival of patients with SHE versus patients without HE; no significant difference was found (p=0.26). Actuarial survival of patients with Child-Pugh A versus Child-Pugh B/C was however highly significant (p=0.002), indicating that the Child-Pugh score is a much better prognostic marker for cirrhosis than SHE. Cox regression analysis was used to estimate the independent effect of Child-Pugh score, age, presence of SHE and clinical hepatic encephalopathy in history on survival. This analysis showed, that the Child-Pugh score is the only prognostic factor with respect to survival (RR for Child-Pugh score: 13 (CI: 2.4 - 70.5; p=0.003), age: 0.9 (CI: 0.15 - 4.9; p=0.86), SHE: 1.2 (CI: 0.24 - 6.3; p=0.80) and clinical HE in history: 0.9 (CI: 0.16 - 5.2; p=0.93)).

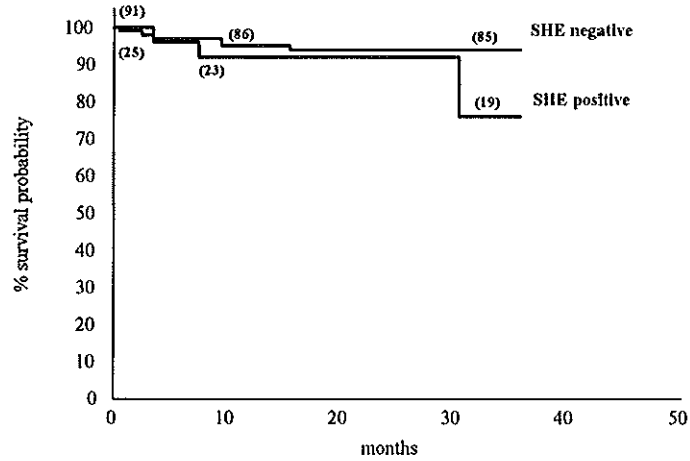


Figure 2 Survival probability of cirrhotic patients with and without SHE (p=0.26: logrank test)

Table 3 Relative risk of child-pugh B/C, clinical hepatic encephalopathy in history and the diagnosis SHE at entry on clinical hepatic encephalopathy during follow-up

Entry feature	Clinical hepatic encephalopathy during follow-up		
	Relative Risk	95% CI	p-value
SHE (positive vs. negative)	3.7	1.2 - 11	0.02
Child-Pugh (B/C vs. A)	19.3	4.1 - 90.8	<0.001
Clinical HE in history (present vs. absent)	0.67	0.2 - 2.2	0.5

Discussion

Interest in the syndrome of subclinical hepatic encephalopathy has increased markedly in the last decade. Many diagnostic tests have been proposed to diagnose this condition. However, the prognostic significance of SHE is largely undefined. In this study we found that patients with SHE have a 3.7 times increased risk to develop clinical hepatic encephalopathy. However, the Child-Pugh score is superior to SHE in predicting the development of clinical hepatic encephalopathy. Survival analysis showed, that the Child Pugh score is the only independent predictor for liver related deaths. Thus, in order to estimate overall prognosis of patients with cirrhosis, the Child-Pugh score should be used.[21-23]

How reliable are our findings?

We studied the prognostic significance of SHE in a large sample of patients with cirrhosis (n=116) and with a follow-up of 29 months. Methods for diagnosing SHE vary; therefore the outcome may be related to the way the patient population was divided. However, the prevalence of SHE in this cohort of patients was 22% and SHE was more often diagnosed in older patients with more advanced liver cirrhosis, which is comparable with other studies.[6,8,11,13] Also, the outcome variable clinical hepatic encephalopathy is defined in various ways. No differences were found between the groups with endogenous and precipitated episodes of clinical hepatic encephalopathy with respect to SHE and clinical hepatic encephalopathy in history; therefore, all episodes of clinical hepatic encephalopathy were pooled. On the basis of our results, we can conclude that patients with SHE are prone to develop clinical hepatic encephalopathy. SHE appears to be a latent form of chronic encephalopathy; in this stage exacerbations of liver disease or extrahepatic complications lead to episodes of clinical hepatic encephalopathy.

What do our findings imply for the cirrhotic patient with SHE?

Overall, a cirrhotic patient with SHE has an increased risk to develop clinical hepatic encephalopathy. The risk is highest in older cirrhotics with Child-Pugh B/C and with esophageal varices. Preventive treatment for clinical hepatic encephalopathy in patients with SHE should be evaluated, especially if daily functioning is affected.[11] The principal factor for increasing survival in cirrhosis is to prevent progression of liver disease by eliminating the etiological factor or by liver transplantation.

References

1. Opolon P, Rapin JR, Huguet C, Granger A, Delorme ML, Boschat M, Sausse A. Hepatic failure coma treated by poyacrylonitrile membrane hymodialysis. Am Soc Artif Intern Organs 1976;22:701-11.
2. Parsons-Smith BG, Summerskill WHJ, Dawson AM, Sherlock S. The electro-encephalograph in liver disease. Lancet 1957;2:867-71.
3. Zeegen R, Drinkwater JE, Dawson AM. Method for measuring cerebral dysfunction in patients with liver disease. Br Med J 1970;2:633-6.
4. Mullen KD, Gacad R. Hepatic encephalopathy. The Gastroenterologist 1996;4:188-202.
5. Gitlin N. Subclinical Portal-Systemic Encephalopathy. Am J Gastroenterol 1988;82:8-11.
6. Rikkers L, Jenko P, Rudman D, Freides D. Subclinical hepatic encephalopathy: detection, prevalence, and relationship to nitrogen metabolism. Gastroenterology 1978;75:462-9.
7. Yen CL, Liaw YF. Somatosensory evoked potentials and number connection test in the detection of subclinical hepatic encephalopathy. Hepatogastroenterol 1990;37:332-4.
8. Quero JC, Hartmann IJC, Meulstee J, Hop WCJ, Schalm SW. The diagnosis of subclinical hepatic encephalopathy in patients with cirrhosis using neuro-psychological tests and automated electroencephalogram analysis. Hepatology 1996;24:556-60.95.
9. Weissenborn K, Scholz M, Hinrichs H et al. Neurophysiological assessment of early hepatic encephalopathy. Electroenceph Clin Neurophysiol 1990;75:289-95.
10. Quero JC, Schalm SW. Subclinical hepatic encephalopathy. Sem Liver Disease 1996;16:321-8
11. Groeneweg M, Quero JC, Essink-Bot ML, Hop WCJ, Schalm SW. The effect of subclinical hepatic encephalopathy on the quality of life. In: Record CO, Al Mardini H editors. Advances in hepatic encephalopathy and metabolism in liver disease. Ipswich, UK: Ipswich Book Company; 1997. P. 311-7.
12. Tarter RE, Hegedus AM, van Thiel DH et al. Nonalcoholic cirrhosis associated with neuropsychological dysfunction in the absence of overt evidence of hepatic encephalopathy. Gastroenterology 1984;1421-7.
13. Gilberstadt SJ, Gilberstadt H, Zieve L, Buegel B, Collier RO, McClain CJ. Psychomotor performance defects in cirrhotic patients without overt encephalopathy. Arch Intern Med 1980;140:519-21.
14. De Franchis R, Pascal JP, Ancona E, Burroughs AK, Henderson M, Fleig W. Definition, methodology and therapeutic strategies in portal hypertension. J Hepatol 1992;15:256-61.
15. Van der Rijt CCD, Schalm SW, De Groot GH, De Vlieger M. Objective measurement of hepatic encephalopathy by means of automated EEG analysis. Electroenceph Clin Neurophysiol 1984;57:423-6.
16. Reitan RM. Validity of the trail making test as an indication of organic brain damage. Percept Mot Skills 1958;8:271-6.
17. Schönleber R. Standardisierung eines psychometrischen Tests zur Erfassung der hepatischen encephalopathie. Thesis. Tübingen, Germany, 1989.
18. Jasper HH. The ten twenty electrode system of the international federation. Electroenceph Clin Neurophysiol

1958;10:371-5.

19. Pugh RNH, Murray-Lyon IM, Dawson JL,Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646-9.
20. Tarter RE, Sandford SL, Hays AL, Carra JP. Hepatic injury correlates with neuropsychologic impairment. Intern J Neurosc 1989;44:75-82.
21. Infante-Rivard C, Esnaola S, Villeneuve JP. Clinical and statistical validity of conventional prognostic factors in predicting short-term survival among cirrhotics. Hepatology 1987;7:660-4.
22. Ginés P, Quitero E, Arroyo V, Ters J, Bruguera M, Rimola A, Caballeria J, Rodes J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. Hepatology 1987;7:122-8.
23. Adler M, Verset D, Bouhdid H, Bourgeois N, Gulbis B, Le Moine O, Van de Stadt J, Gelin M, Thiry Ph. Prognostic evaluation of patients with parenchymal cirrhosis: proposal for a new simple score. J Hepatol 1997;26:642-9.

Discussion and Conclusions

In 1994, when the investigations summarized in this thesis started, unresolved issues in hepatic encephalopathy were: the pathophysiology and subclinical hepatic encephalopathy. At the end of 1998 the pathophysiology remains largely unresolved; however, the issue of subclinical hepatic encephalopathy has been clarified for the major part.

Pathophysiological aspects

In this thesis several aspects of the pathophysiology of HE were subject to investigation: ammonia-induced alterations in glutamate neurotransmission, structural brain damage, the GABA-benzodiazepine receptor complex and thyroid hormone metabolism.

An intervention study with the NMDA-receptor antagonist MK-801 indicated, that glutamate toxicity has no causative relation with the pathophysiology, since blocking of post-synaptic NMDA receptors does not reduce symptoms of HE, nor does it affect survival. Despite these results, glutamate neurotransmission remains a topic for future research: Firstly, because experiments with another glutamate antagonist (memantine) showed improvement in symptoms of HE in rats with acute liver failure and ammonia infusion; [1] probably, memantine is much better tolerated than MK-801. Secondly, our observation, that blocking the post-synaptic NMDA receptor induces symptoms of HE in control animals, and enhances the development of encephalopathy in animals with acute liver failure and acute hyperammonemia suggests, that decreased, rather than increased glutamate neurotransmission could have a role in the pathophysiology. Modulation of post-synaptic NMDA-receptors by excess extracellular glutamate could be an explanation for this observation.[2]

HE is still believed to be a reversible neuropsychiatric syndrome. [3] However, studies on autopsied brain tissue of cirrhotic patients who died in hepatic coma has shown selective loss of glial fibrillary acidic protein (GFAP) in grey matter and Alzheimer type II gliosis. [4,5] In addition, this selective decrease in GFAP can be induced in cultured astrocytes, by exposing them to ammonia [6]. In the study on neuronal and glial marker protein low molecular NF68 breakdown products in the hippocampus of rabbits with hyperammonemia and with acute liver failure were observed, suggesting some structural changes in the brain were actually present. Hypothetically, the experimental models used may have had a too limited survival time for extensive structural brain damage to become detectable. Structural brain damage in HE remains a topic, especially since both neurophysiological and neuropsychological deficits remain present in some patients after liver transplantation (unpublished observations).

From the very first moment it came to light, the GABA-benzodiazepine hypothesis has been generally well received. However, controlled trials with benzodiazepine antagonists showed no major effect on hepatic encephalopathy, except if encephalopathy is precipitated by exogenous benzodiazepine use. Endogenous benzodiazepines play no major role in the pathophysiology of hepatic encephalopathy, and flumazenil is only indicated in patients with cirrhosis and encephalopathy precipitated by benzodiazepine use.

Does our finding mean that the GABA-benzodiazepine hypothesis can be discarded?

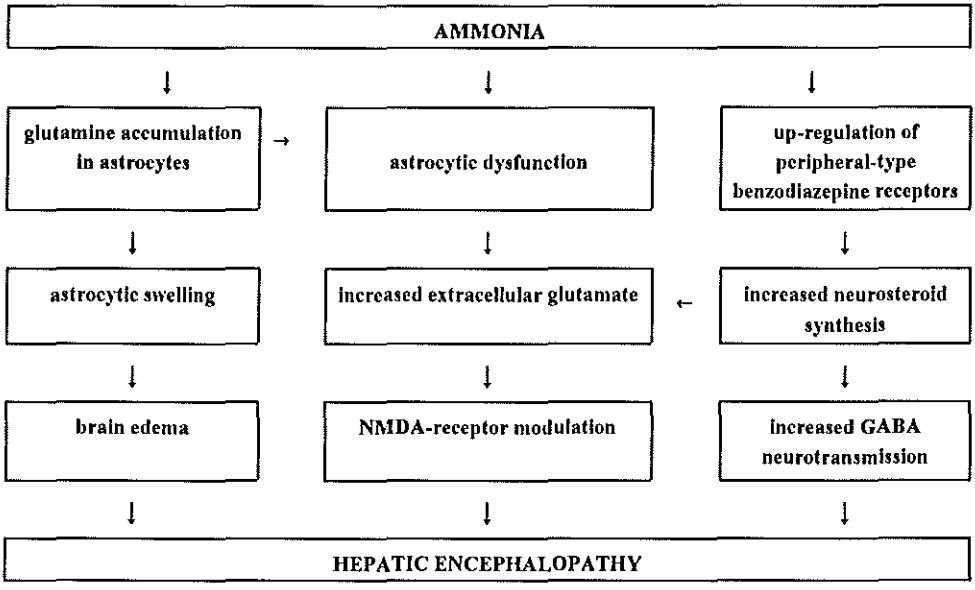
The answer is no. Studies with the partial inverse agonist sarmazenil in a dog model of chronic HE showed improvement in clinical signs of HE and in mean dominant frequency of the EEG; flumazenil was not effective in the same dog model.[7] These results cannot be explained by endogenous benzodiazepine-like substances, nor by exogenous benzodiazepine use. Probably, sarmazenil modulates the GABA-receptor directly, [8] or it interferes with peripheral-type benzodiazepine repectors. [9] Peripheral-type benzodiazepine receptors are located on the outer membrane of mitochondria of astrocytes and microglia. Binding to these receptors leads to enhanced production of neurosteroids (e.g. pregnenolone, tetrahydroprogesterone) by facilitating the transport of cholesterol. In experimental models of HE these receptors are up-regulated. This enhanced neurosteroid synthesis is of interest, because these substances have a extremely potent positive modulatory effect on GABA receptors. In addition, these neurosteroids inhibit the uptake of GABA and glutamate into astrocytes. [10] Future research on GABA neurotransmission within the area of the pathophysiology of HE should especially be directed to the peripheral benzodiazepine antagonists and their effect on neurosteroid synthesis.

A case control study, performed at our department, showed that triiodothyronine (T_3) has strong predictive power for HE.[11] In addition, a case control study suggested, that supplementation of thyroid hormone may improve portal-systemic encephalopathy. [12] The study on thyroid hormone metabolism included in this thesis does not solve the issue of altered thyroid hormone metabolism and its implications for the pathophysiology of HE. In non-thyroidal illness, the uptake of T_4 in the brain may be affected by the same inhibitory substances that compromise uptake into hepatocytes.[13,14] The concept of "local brain hypothyroidism" as factor in the pathophysiology of hepatic encephalopathy should be a topic for future research, especially because thyroid hormones affect postsynaptic glutamate - and both central and peripheral benzodiazepine receptor function. [15-17]

Of the three other potential fields in the pathophysiology of HE mentioned in the introduction, glutamine accumulation is the most consistent finding. Glutamine is synthesized in astrocytes out of ammonia and glutamate by the enzyme glutamine synthetase. This is the only pathway in the brain for ammonia detoxification.[18 - 20] Glutamine accumulation results in astrocytic swelling and brain edema formation. This concept is supported by the finding, that blocking glutamine synthetase with methionine sulfoximine prevents brain swelling in rats after portacaval anastomosis. [21 -22] However, methionine sulfoximine induces seizures in these animals, probably because glutamate is not converted to glutamine and accumulates extracellularly. [21] As a concept for future studies in experimental hyperammonemia and acute liver failure, both blocking glutamine synthesis and at the same time blocking post-synaptic NMDA receptors could learn us more about the role of both glutamine and glutamate in the pathophysiology of HE.

Figure 1 summarizes the three major mechanisms potentially involved in the pathophysiology of HE, that could be focus of future investigations.

Figure 1



Subclinical hepatic encephalopathy

The area of SHE can be divided in diagnosis, implications, and therapy. With regard to the diagnosis, the problem was how to recognize SHE. Applying time-consuming neuropsychological and neurophysiological test batteries to each cirrhotic patient at the outpatient department is not feasible. In addition, in the standard tests advocated previously, [23-26] normal values were not corrected for age, which resulted in a considerable number of false positives. [27] At our department, a combination of psychometric tests (Number Connection test A and Symbol Digit Test; both with age-corrected normal values) and one neurophysiological test (automated EEG analysis) was used. SHE was defined present, if at least one psychometric test was abnormal and/or if there was abnormal slowing of the EEG. Applying these criteria to an unselected group of 137 patients with cirrhosis showed a prevalence of SHE of 23 %. In addition, the prevalence of SHE increased significantly from 14 % of patients with Child-Pugh A cirrhosis, to 45 % of patients with Child-Pugh B or C cirrhosis. [28]

Separate analysis of the Sickness Impact Profile yielded five statements, which can be used for screening the outpatient population for SHE. After initial screening, a subgoup of patients with a high probability of SHE can thus be selected for more specific testing: either with psychometric tests or neurophysiological tests. The types of specific tests should in our opinion be at least one psychometric test and one neurophysiological test, provided that normal values corrected for age are available. Under these conditions, the precise type of specific test used will be dependent on local practice.

The first study addressing the clinical implications of SHE postulated, that the psychomotor defects found in patients with SHE had a negative effect on the fitness to drive. [29] In this study, 40 cirrhotic patients underwent extensive neuropsychological testing (24 tests, 102 variables studied !). On the basis these tests, the authors concluded, that 60 % of the cirrhotics were unfit to drive a car and that the driving capacity of 25 % was questionable. [29] Unfortunately, the investigators do not describe the method used to assess driving fitness. This initial concept on driving capacity and SHE was however not confirmed by recent studies. [30, 31] In the latter study, the driving capacity of 9 cirrhotic patients with SHE was tested, using a driving simulator and a real driver's examination "on the road". Results indicated, that patients with SHE did not drive less well when compared with 15 healthy control subjects. [31]

We decided to apply a quality of life questionnaire to evaluate the clinical implications of SHE. Results indicate, that daily function is significantly impaired in patients with SHE. In addition, separate analysis of the statements of the Sickness Impact Profile questionnaire yielded five statements on daily life specifically directed to SHE. These five statements, together with clinical variables, are now used to select cirrhotic patients with a high probability of SHE (see above).

Another clinical implication of the diagnosis SHE refers to its natural history. It has been suggested in literature, [25, 32] that SHE may be a prelude to clinically overt HE. However, long-term follow-up studies of cirrhotic patients with SHE were not available. The results of our prognostic study indicate, that patients with SHE have an increased risk to develop clinical HE, but that prognosis is determined by the Child-Pugh score.

Lastly, one major issue on SHE remains unresolved: Can SHE be treated? Several intervention studies using nonabsorbable saccharides, [33 - 36] dietary manipulation, [25, 37] and branched-chain amino acids (BCAA) [38, 39] have been performed, all of which document some improvement in SHE. Reliable interpretation of these studies is hampered by differences in the populations studied, the diagnostic criteria used and by learning effects in the psychometric tests. [27] At our own department, a placebo-controlled trial with low-dose lactulose for six months in cirrhotic patients without HE and hyperammonemia (arterial ammonia above 30 µmol/L) showed no effect on arterial ammonia levels, psychometric tests, nor on the EEG. Interestingly, a significant improvement in the psychosocial subscore of the SIP was found in both experimental groups. The investigators suggest, that a higher dose of lactulose could be effective in cirrhotic patients with a confirmed diagnosis of SHE and an impaired quality of life. [40]

To fully estimate the efficacy of treatment strategies for SHE, symptoms of SHE should be the principal outcome measure, and psychometric tests or neurophysiological tests should be secondary outcome measures.

CONCLUSIONS

1. Glutamate toxicity has no major role in the pathophysiology of hepatic encephalopathy.

2. Encephalopathy as a result of acute liver failure and acute hyperammonemia is not associated with structural changes in the brain,

3. Treatment with the benzodiazepine antagonist flumazenil indicates, that endogenous benzodiazepines have no major role in the pathophysiology of hepatic encephalopathy; flumazenil is indicated only if encephalopathy is precipitated by benzodiazepines use,

4. Low triiodothyronine and thyroxine levels in dogs with portal-systemic encephalopathy indirectly suggest, that brain uptake of these substances is also affected.

5. Automated electroencephalogram analysis to diagnose (subclinical) hepatic encephalopathy can be performed at random hours during the day,

6. Subclinical hepatic encephalopathy has a significant adverse effect on daily functioning,

7. Cirrhotic patients can be screened for subclinical hepatic encephalopathy, using a symptom checklist of five statements in combination with clinical variables. The diagnosis should be confirmed by psychometric tests and automated EEG analysis,

8. Patients with subclinical hepatic encephalopathy have an increased risk to develop clinically manifest hepatic encephalopathy. The Child-Pugh score is superior to subclinical hepatic encephalopathy with respect to prognosis.

References

1. Vogels BAPM, Maas MAW. Memantine, a noncompetitive NMDA receptor antagonist improves hyperammonemia-induced encephalopathy and acute hepatic encephalopathy in rats. Hepatology 1997;25:820.
2. Bustos G, Abarca J, Forray MI, Gysling K, Bradberry CW, Roth RH. Regulation of excitatory amino acid release by N-methyl-D-aspartate receptors in rat striatum: in vivo microdialyis studies. Brain Res 1992;585:105-15.
3. Sherlock S. Diseases of the liver and biliary system. (8th ed.), Blackwell Scientific Publications, Oxford, England, 1989, pp. 95-115.
4. Kretzschmar HA, DeArmond SJ and Forno LS. Measurement of GFAP in hepatic encephalopathy by ELISA and transblots. J Neuropathol Exp Neurol 1985;4: 459-471.
5. Adams RD and Foley JM. The neurological disorder associated with liver disease. In: Merritt HH, Hare CC. (eds.), Proc Ass Res Nerv Ment Dis 1953;32: 198-237.
6. Norenberg MD, Neary JT, Norenberg LOB, McCarthy M. Ammonia induced d2ecrease in glial fibrillary acidic protein in cultured astrocytes. J Neuropathol Exp Neurol 1990;49:399-405.
7. Meyer HP, Legemate DA, van den Brom WE, Rothuizen J. Improvement of chronic encephalopathy in dogs by the benzodiazepine receptor partial invers agonist sarmazenik but not by the antagonist flumazenil. Metab Brain Dis 1998: in press.
8. Costa E. The allosteric modulation of $GABA_A$ receptors. Seventeen years of research. Neuropharmacology 1991;4:225-35.
9. Haefely WE. The $GABA_A$ benzodiazepine receptor: biology and pharmacology. Neurobiol Axiety 1990;3:165-88.
10. Norenberg MD. The peripheral-type benzodiazepine receptor and neurosteroid synthesis in hepatic encephalopathy (abstract). International Symposium: Hepatocerebral disorders: pathophysiology of cerebral dysfunction and new approaches to diagnosis and therapy. La Sapinière, Val David, Quebec, Canada, September 20-23, 1997.
11. Van der Rijt CCD. Hepatic encephalopathy: clinical and experimental studies [thesis]. Erasmus Univ.; 1991.
12. Hitoshi S, Terao Y, Sakuta M. Portal-systemic encephalopathy and hypothalamic hypothyroidism: effect of thyroid hormone on ammonia metabolism (case report). Int Med 1993;32:655-658.
13. Visser TJ. Sulfation and glucuronidation pathways of thyroid hormone metabolism. In: Wu S-Y, Visser TJ Eds. Thyroid hormone metabolism: molecular biology and alternate pathways. CRC Press, Boca Raton, USA, p.85.
14. Laksmanan M, Goncalves E, Lessly G, Foti D, Robbins J. The transport of thyroxine into mouse neuroblastoma cells, NB41A3: the effect of L-system amino acids. Endocrinology 1990;126:3245-50.
15. Oguro K, Ito M, Tsuda H, Mutoh K, Shiraishi Y, Mikawa H. Interactions of thyroid hormones with L-(^{3}H)glutamate binding sites, with special reference to N-methyl-D-aspartate receptors. Res Commun Chem Pathol Pharmacol 1989;65:181-96.
16. Medina JH, De Robertis E. Benzodiazepine receptor and thyroid hormones: in vivo and in vitro modulation. J Neurochem 1985;44:1340-44.
17. Gavish M, Weizman A, Okun F, Youdim MBH. Modulatory effect of thyroxine treatment on central and peripheral benzodiazepine receptors in the rat. J Neurochem 1986;47:1106-10.
18. Tossman U, Delin A, Eriksson LS, Ungerstedt U. Brain cortical amino acids measured by intracerebral dialysis in portacaval shunted rats. Neurochem Res 1987;12:265.
19. Butterworth RF. Hepatic encephalopathy and brain edema in acute hepatic failure: does glutamate play a role ? (editorial). Hepatology 1997;25:1032-34.

20. De Knegt RJ, Schalm SW, van der Rijt CCD, Fekkes D, Daim E, Hekking I. Extracellular brain glutamate during acute liver failure and during acute hyperammonemia simulating acute hepatic failure: an experimental study based on in vivo brain dialysis. J Hepatol 1994; 20: 119-26.
21. Takahashi H, Koehler RC, Brusilow SW, Traystman RJ. Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. Am J Physiol 1991;261:H825-29.
22. Blei AT, Olafsson S, Therrien G, Butterworth RF. Ammonia-induced brain edema and intracranial hypertension in rats after portacaval anastomosis. Hepatology 1994;19:1431-44.
23. Conn HO. Subclinical hepatic encephalopathy. In: Hepatic Encephalopathy: Syndromes and Therapies. Conn HO, Bircher J eds. Bloomington, Illinois: Medi-Ed Press, 1994:27-39.
24. Gitlin N, Lewis DC, Hinkley L. The diagnosis and prevalence of subclinical hepatic encephalopathy in apparently healthy ambulant, non-shunted patients with cirrhosis. J Hepatol 1986; 3: 75-82.
25. Rikkers L, Jenko P, Rudman D, Freides D. Subclinical hepatic encephalopathy: detection, prevalence, and relationship to nitrogen metabolism. Gastroenterology 1978; 75: 462-469.
26. Schomerus H, Schreiegg J. Prevalence of latent portasystemic encephalopathy in an unselected population of patients with liver cirrhosis in general practice. Z Gastroenterol 1993; 31: 231-234.
27. Quero JC, Schalm SW. Subclinical hepatic encephalopathy. Seminars in Liver Disease 1996;16:321-8.
28. Quero JC, Hartmann IJC, Meulstee J, Hop WCJ, Schalm SW. The diagnosis of subclinical hepatic encephalopathy in patients with cirrhosis using neuropsychological tests and automated electroencephalogram analysis. Hepatology 1996; 24:556-60.
29. Schomerus H, Hamster W, Blunck H, Reinhard U, Mayer K, Döll W. Latent portasystemic encephalopathy. I. Nature of cerebral functional defects and their effect on fitness to drive. Dig Dis Sci 1981;26:622-30.
30. O'Neill D. Physicians, elderly drivers and dementia. Lancet 1992;41-43.
31. Srivastava A, Mehta R, Rothke Sp, Rademaker AW, Blei AT. Fitness to dirve in patients with cirrhosis and portal-systemic shunting: a pilot study evaluating driving performance. J Hepatol 1994;21:1023-28.
32. Yen CL, Liaw YF. Somatosensory evoked potentials and number connection test in the detection of subclinical hepatic encephalopathy. Hepatogastroenterol 1990; 37: 332-334.
33. McClain CJ, Potter TJ, Kromhout JP, Zieve L. The effect of lactulose on psychomotor performance tests in alcoholic cirrhotics without overt hepatic encephalopathy. J Clin Gastroenterol 1984;6:325-29.
34. Morgan MY, Alonso M, Stanger LC. Lactitol and lactulose for the treatment of subclinical hepatic encephalopathy in cirrhotic patients. J Hepatol 1989;8:208-17.
35. Salerno F, Moser P, Maggi A, Vitaliani G, Benetti G. Effects of long-term administration of low-dose lactitol in patients with cirrhosis but without overt encephalopathy. J Hepatol 1994;21:1092-96.
36. Watanabe A, Sakai T, Sato S, Imai F, Ohto M, Arakawa Y. Clinical efficacy of lactulose in cirrhotic patients with and without subclinical hepatic encephalopathy. Hepatology 1997;26:1410-14.
37. De Bruijn KM, Blendis LM, Zilm DH, Carlen PL, Anderson GH. Effect of dietary protein manipulations in subclinical portal-systemic encephalopathy. Gut 1983;24:53-60.
38. Egberts EH, Schomerus H, Hamster W, Jürgens P. Branched chain amino acids in the treatment of latent portosystemic encephalopathy. Gastroenterology 1985;88:887-95.
39. Plauth M, Egberts EH, Hamster W, Török M, Müller PH, Brand O, Fürst P, Dölle W. Long-term treatment of latent portosystemic encephalopathy with branched-chain amino acids. A double-blind placebo-controlled crossover study. J Hepatol 1993;17:308-14.
40. Quero JC, Groeneweg M, Meulstee J, Hop WCJ, Schalm SW. Does low-dose lactulose improve quality of life in patients with liver cirrhosis ? In: advances in Hepatic Encephalopathy and Metabolism in Liver Disease. Ninth International Symposium on Ammonia, 1996, ch. 64:pp.459-65.

Summary / Samenvatting

In this thesis several aspects of the pathophysiology of HE were subject to investigation: ammonia-induced alterations in glutamate neurotransmission, structural brain damage, the GABA-benzodiazepine receptor complex and thyroid hormone metabolism;

In **chapter 1.1** results are presented of measurement of neuronal (NF-68, NF-200, NSE) and glial marker proteins (GFAP, S-100) in the brain of rabbits with acute liver failure and acute hyperammonemia. Increased proteolysis of these marker proteins reflects structural brain damage. The content of neuronal and glial cell markers was not affected by either of the two experimental conditions. However, low molecular weight proteolytic fragments of the NF 68 kD polypeptide were observed in the hippocampus of three out of six animals in both experimental conditions, indicating minor neurofilament degeneration. These findings indicate, that no extensive structural brain damage occurs in the cours of acute liver failure and acute hyperammonemia.

In **chapter 1.2** the implication of increased extracellular brain glutamate levels on the pathophysiology of HE was tested. In a placebo-controlled experiment, the efficacy of the glutamate antagonist MK-801 was evaluated in rabbits with acute liver failure and acute hyperammonemia. A dose of MK-801 of 0.05 ml/kg/hr enhanced the development of HE in both experimental groups, and induced encephalopathy in the control rabbits. Survival was not affected by MK-801 treatment. The results do not give support to the hypothesis, that increased extracellular glutamate has a causative role in the pathophysiology of HE.

In **chapter 1.3** alterations in thyroid hormone metabolism in HE was evaluated. In dogs with a portacaval shunt and partial hepatectomy serum levels of triiodothyronine (T3) thryoxine (T4), free T4, rT3, T4-sulphate and T3-sulphate were measured it baseline and at four weeks after surgery. PCS dogs developed clinical signs of HE, hyperammonemia and hypalbuminemia after four weeks. T4 and T3 levels decreased significantly, and T4-sulphate degradation was significantly diminished .
Free T4 , reverse-T3 and T3-sulphate levels remained unaltered. HE is accompanied by low T4 and T3 levels and normal free T4 levels. Low serum levels of T3 could be the result of decreased hepatic synthesis (diminished D1 activity), decreased hepatic uptake of T4 or by diminshed plasma binding of both T3 and T4. It is suggested, that specific inhibitors of plasma binding of thyroid hormone - like bilirubin and L-amino acids - also compromise hepatic uptake of T4. In addion, the same inhibitory substances could affect brain uptake of T4, which could have role in the pathophysiology of HE.

In **chapter 1.4** the efficacy of the benzodiazepine antagonist flumazenil was assessed clinically and electrophysiologically in a double blind, randomized, placebo-controlled multicentre study in patients with HE. Patients who were positive for benzodiazepines were excluded. This study primarily focussed on the effect on the EEG, next to clinical improvement

of HE. EEG gradings of five out of 17 (29%) flumazenil treated patients showed an improvement in EEG-grading compared to two out of 15 (13%) placebo treated patients. In conclusion, endogenous benzodiazepine have no major role in the pathophysiology of HE. Flumazenil is indicated only, if encephalopathy is precipitated by exogenous benzodiazepines.

The four studies on subclinical hepatic encephalopathy (SHE) included in this thesis focussed on the diagnosis and the implications of the syndrome;

In **chapter 2.1** a study is presented, in which the variability in the EEG and the effect of meals and physical exercise was evaluated in patients with cirrhosis without HE, with SHE and with HE. Therefore, 12 hour portable EEG registration and repetitive automated analysis of the EEG (spectral analysis) was performed. EEG recordings were also graded by visual inspection. No significant differences in mean dominant frequency (MDF) and percentage theta activity were observed during the morning, after lunch, after exercise, or after dinner. On the basis of visual inspection slowing of the EEG was observed after lunch in the majority of patients with SHE and in half of patients with clinically manifest HE. Assessment of patients with cirrhosis by EEG is hardly influenced by time of the day, meals or exercise, when assessed by MDF and percentage theta activity. EEG grading by visual inspection is a more sensitive method to detect variability in the EEG. However, changes in consciousness during the day reported by patients with cirrhosis rather reflect physiological variability than aggravation of HE. Therefore, automated EEG analysis can be performed at random hours during the day.

In **chapter 2.2** the influence of SHE on patients' daily functioning was assessed using the Sickness Impact Profile (SIP). Patients with SHE reported significantly more impairments in all 12 scales of the SIP, in the psychosocial subscore, the physical subscore, as well as in the total SIP score, compared to cirrhotic patients without SHE. Multivariate analysis taking into account severity of liver disease (Child-Pugh score), presence of varices and alcoholic etiology showed that SHE independently was related to a diminished total SIP score.

In **chapter 2.3** an effort was made to determine which statements of the SIP have predictive power for SHE. Therefore, patients with and without SHE were compared for differences in response to all statements of the SIP. This analysis yielded thirty-six statements that were significantly more often true for patients with SHE. After multivariate analysis, five statements of the SIP (related to alertness, sleep and rest, fine motor skills and work) showed to have independent predictive power for SHE, together with the patient characteristics: Child-Pugh score, oesophageal varices and male sex. An SHE score was constructed, containing the five statements and the patient characteristics. With this score the probability for SHE can be assessed, making it a bedside screening test for SHE.

In **chapter 2.4** the prognostic significance of SHE with regard to the development of HE and survival was evaluated. Patients with SHE were older, had a higher Child-Pugh score, more often had esophageal and/or gastric varices and episode(s) of clinical HE in history. During a median follow-up of 29 months (range 1-49 months) patients with SHE significantly more often had episodes of clinical HE; survival however, was similar to patients without SHE, and was mainly determined by the Child-Pugh score. The Child-Pugh score was also superior to SHE in predicting episodes of clinical HE. The prognostic significance of SHE appears limited.

In the **Discussion and Conclusions** chapter the significance of the studies on the pathophysiology are discussed, and three major mechanisms potentially involved in the pathophysiology are presented: glutamine accumulation in the brain and its role in cerebral edema formation, astrocyte dysfunction, altered glutamate neurotransmission and the role of increased neurosteroid synthesis in GABA neurotransmission. Future research should focus on these three mechanisms.The issue of subclinical hepatic encephalopathy (SHE) has been resolved for the major part. The diagnosis, implications and therapeutic options for SHE are discussed. With the proposed SHE score an initial screening test for SHE is presented, with which SHE can be diagnosed on the basis of symptoms related to daily functioning. Therapeutic options of SHE should be evaluated in the future, in which symptoms of SHE should be the primary endpoint.

In dit proefschrift zijn verschillende aspecten van de pathofysiologie van hepatische encephalopathie (HE) belicht: ammoniak-gemedieerde veranderingen in glutamaat neurotransmissie, structurele hersenbeschadiging, de rol van het GABA-benzodiazepine receptor complex en het schildklierhormoon metabolisme;

In **hoofdstuk 1.1** zijn de resultaten gepresenteerd van metingen van specifieke neuronale (NF-68, NF-200, NSE) en gliale (GFAP en S-100) eiwitten in de hersenen van konijnen met acute leverinsufficiëntie en acute hyperammoniëmie. Verhoogde afbraak van deze eiwitten zijn een teken van hersenbeschadiging. De totale inhoud van de neuronale en gliale eiwitten bleef onveranderd in beide experimentele modellen. In de hippocampus van drie van de zes konijnen in beide groepen werden echter laag-moleculaire eiwitfragmenten van het NF-68 gevonden. Deze bevining duidt op beginnende hersenbeschadiging. De resultaten geven aan, at er geen sprake is van uitgebreide hersenbeschadiging in het beloop van acute leverinsufficiëntie en acute hyperammoniëmie.

In **hoofdstuk 1.2** werd in een placebo-gecontroleerd experiment het effect van de glutamaat antagonist MK-801 geëvalueerd op tekenen van encephalopathie en overleving van konijnen met acute leverinsufficiëntie en acute hyperammoniëmie. Een dosering van MK-801 van 0.05 mg/kg/uur versnelde het ontwikkelen van encephalopathie in beide experimentele modellen, en induceerde encephalopathie in controle konijnen. De overleving werd niet beïnvloed door MK-801. De resultaten geven aan, dat verhoogde extracellulaire levels van glutamaat geen oorzakelijke relatie heeft met de pathofysiologie van HE.

In **hoofdstuk 1.3** werden veranderingen in het schildklierhormoon metabolisme onderzocht. In honden met een portacavale shunt en een partiële leverresectie werden aan het begin van het experiment en vier weken na de operatie de serumwaarden van triiodothyronine (T3), thyroxine (T 4), vrij T4, reverse-T3, T4-sulfaat en T3-sulfaat bepaald. De honden hadden vier weken na de operatie allen teken van encephalopathie, hyperammoniëmie en hypalbuminemie. T4 en T3 waren significant verlaagd en de afbraak van T4-sulfaat was significant afgenomen. Vrij T4, rT3 en T3-sulfaat bleven gelijk. De resultaten geven aan, dat HE gepaard met een laag T4 en T3. Laag T3 kan een gevolg zijn van verminderde synthese in de lever (verminderde D1 activiteit), verminderde opname van T4 in de lever of door verminderde eiwitbinding van T3 en T4. De suggestie is, dat specifieke remmers van de plasma binding van schildklierhormoon - zoals bilirubine en L-aminozuren - ook de opname van T4 in de lever remmen. Bovendien kunnen dezelfde remmers verantwoordelijk worden geacht voor een verminderde opname van T4 in de hersenen, hetgeen van betekenis kan zijn voor de pathofysiologie van HE.

In **hoofdstuk 1.4** is de effectiviteit van de benzodiazepine antagonist flumazenil onderzocht in een dubbel-blind, placebo-gecontroleerd multicentrische studie in patiënten met HE. In deze studie werd met name gekeken naar verbetering van het electroencephalogram (EEG), naast klinische verbetering van HE. Bij alle patiënten was het gebruik van benzodiazepines uitgesloten. Het EEG verbeterde bij vijf van de zeventien (29%) patiënten, vergeleken met twee van de vijftien patiënten, behandeld met placebo. Klinische verbetering werd waargenomen bij drie van de vijf behandelde patiënten met een EEG verbetering. De conclusie is, dat endogene benzodiazepines geen grote rol spelen in de pathofysiologie van HE, en dat behandeling met flumazenil alleen geïndiceerd is, als er sprake is van benzodiazepine gebruik.

De vier studies over subklinische hepatische encephalopathie (SHE) die onderdeel vormen van dit proefschrift, richten zich op de diagnose en de betekenis van het syndrome;

In **hoofdstuk 2.1** wordt een studie gepresenteerd, waarin de variabiliteit van het EEG en de invloed van maaltijden en lichaamsbeweging onderzocht is bij patiënten met levercirrose zonder HE, met SHE en met HE. Hiertoe werd gedurende 12 uur een ambulante EEG registratie gemaakt en werd met intervallen spectraal analyse verricht van het EEG. Tevens werd het EEG visueel beoordeeld. Er werden geen significante veranderingen in de mean dominant frequency (MDF), noch in het percentage theta gevonden gedurende de ochtenduren, na de lunch, na lichaamsbeweging, en na het avondeten. Op basis van visuele beoordeling van het EEG werd echter EEG vertraging vastgesteld na de lunch in bijna alle patiënten met SHE en in de helft van de patiënten met HE. De conclusie is, dat de evaluatie van (S)HE met behulp van spectraal analyse van het EEG (MDF en percentage theta) nauwelijks beïnvloed wordt door het moment van de dag, maaltijden, noch door de mate van lichaamsbeweging. Evaluatie van het EEG door visuele beoordeling is een gevoeliger methode om variabiliteit in het EEG te detecteren. De veranderingen in bewustzijn, die worden gerapporteerd door patiënten met cirrose gedurende de dag lijken echter eerder een fysiologische variabiliteit te zijn, dan variabiliteit in ernst van encephalopathie. Spectraal analyse van het EEG bij patiënten met levercirrose en (S)HE kan dan ook op elk willekeurig moment van de dag worden uitgevoerd.

In **hoofdstuk 2.2** werd het effect van SHE op het dagelijks functioneren onderzocht met de Sickness Impact Profile (SIP). Patiënten levercirrose en SHE rapporteerden significant meer afwijkingen op alle twaalf schalen van de SIP, op de psychosociale subscore, de fysieke subscore, alsook op de totale SIP score, in vergelijking met cirrose patiënten zonder SHE. Multivariaat analyse met de totale SIP score, de ernst van leverziekte (Child-Pugh score), de aanwezigheid van varices en alcohol als etiologie toonde aan, dat de diagnose SHE onafhankelijk gerelateerd is aan een verhoogde totale SIP score.

In **hoofdstuk 2.3** werd uitgezocht welke beweringen van de SIP vragenlijst voorspellende waarde hebben voor de diagnose SHE. Hiertoe werd bij patiënten met en zonder SHE verschillen in response op alle SIP beweringen afzonderlijk geëvalueerd. 36 van de 136 beweringen van de SIP bleken significant vaker door patiënten met SHE te worden aangekruist. Na multivariaat analyse bleken vijf beweringen (verband houdend met alertheid, slapen en rusten, fijne motoriek, en werk) en de patiënt karakteristieken: Child-Pugh score, aanwezigheid van slokdarmvarices en het manlijk geslacht voorspellende betekenis te hebben voor de diagnose SHE. Op basis van deze analyse werd een SHE score gemaakt, waarmee de kans op SHE kan worden ingeschat. Hiermee kan op eenvoudige wijze voor aanwezigheid van SHE worden gescreend.

In **hoofdstuk 2.4** is de prognostische betekenis van SHE met betrekking tot het ontwikkelen van HE en overleving geëvalueerd. Patiënten met SHE waren ouder, hadden een hogere Child-Pugh score, hadden vaker varices en hadden tevens vaker episoden van HE doorgemaakt in de voorgeschiedenis. Na een gemiddelde follow-up van 29 maanden (range 1 - 49 maanden) maakten patiënten met SHE vaker episoden door van HE. De overleving was niet verschillend tussen patiënten met en zonder SHE en werd bepaald door de Child-Pugh score. De Child-Pugh score was ook superieur boven SHE in het voorspellen van episoden van HE. De conclusie is, dat patienten met SHE een verhoogd risico hebben op het ontwikkelen van HE, maar dat de prognose bepaald wordt door de Child-Pugh score.

In de **Discussie en Conclusies** wordt de betekenis van de studies inzake de pathofysiologie van HE besproken, Tevens worden een aantal mechanismen gepresenteerd, die mogelijk een belangrijke rol spelen in de pathofysiologie: glutamine ophoping in de hersenen en de rol hiervan bij de vorming van cerebraal oedeem, astrocyten dysfunctie, veranderingen in glutamaat neurotransmissie en de rol van verhoogde synthese van neurosteroiden op GABA neurotransmissie.

Tevens worden de diagnose , de betekenis en de behandelingsopties van SHE besproken. Met de voorgestelde SHE score kan SHE worden gediagnostiseerd aan de hand van symptomen, die gerelateerd zijn aan het dagelijks functioneren. Behandelingsopties voor SHE dienen nader te worden geëvalueerd, waarbij deze symptomen als primair eindpunt dienen te worden gebruikt.

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Curriculum Vitae

De auteur van dit proefschrift werd op 12 oktober 1968 geboren in het Dijkzigt Ziekenhuis te Rotterdam. Na het behalen van het V.W.O diploma aan de Christelijke Scholengemeenschap "Comenius", te Capelle aan den IJssel, werd op 1 september 1987 gestart met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Tijdens de studie Geneeskunde werkte de auteur als student-assistent aan de afdeling Maag - Darm en Leverziekten (tevens Inwendige Geneeskunde), sectie Leverziekten en Levertransplantatie (hoofd: Prof. Dr. S.W. Schalm), alwaar de basis voor dit proefschrift werd gelegd. Op 15 juli 1994 werd het arts-examen behaald.
Van 1 september 1994 tot 1 april 1998 werd gewerkt aan dit proefschrift. Gedurende deze periode werd tevens in deeltijd onderzoek verricht aan de subafdeling gastro-enterologie van de afdeling Kindergeneeskunde van het Sophia Kinderziekenhuis te Rotterdam (hoofd: dr. M. Sinaasappel). De werkzaamheden aan deze afdeling betroffen de poliklinische controle van kinderen met hepatitis B, alsmede de coördinatie van een multicentrisch behandelingsprotocol inzake interferon therapie bij kinderen met chronische hepatitis B. Met ingang van 1 april 1998 is Michael Groeneweg werkzaam als assistent-geneeskundige in opleiding tot kinderarts in het Juliana Kinderziekenhuis te Den Haag (opleider: Prof. Dr. AJ van der Heijden).
De auteur is gehuwd met Isis Koolhoven, en heeft een zoon (Ernö).