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Short Communication

Changes in liver function parameters after percutaneous radiofrequency ablation therapy in patients with hepatocellular carcinoma

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Aim: To evaluate changes in liver function parameters and risk factors 1 year after percutaneous radiofrequency ablation (RFA) therapy in patients with hepatocellular carcinoma (HCC).

Methods: Subjects in this retrospective study comprised 45 patients with HCC who underwent RFA therapy (RFA alone, $n = 25$; transcatheter arterial embolization therapy before RFA, $n = 20$) and showed no recurrence of HCC 1 year after RFA. Serial changes in serum total bilirubin, albumin, prothrombin time and Child–Pugh score (CPs) were evaluated before and after RFA. In addition, Cox proportional hazards regression analysis was used to clarify risk factors for aggravation of liver function after RFA therapy.

Results: Serum albumin levels showed a significant decrease from before (3.6 ± 0.4 g/dL) to 12 months after RFA therapy (3.2 ± 0.4 g/dL; $P \leq 0.05$). CPs was significantly increased from before (6.4 ± 1.4) to both 6 months (6.8 ± 1.9; $P \leq 0.05$) and 12 months after RFA (6.9 ± 2.0; $P \leq 0.05$). Based on stepwise multivariate analysis, CPs of 9 or more before RFA was selected as a significant risk factor for long-term aggravation of liver function after RFA.

Conclusion: Liver function parameters, particularly serum albumin level, gradually and dominantly decreased in HCC patients with grade B and C according to the CPs classification over the course of 1 year after RFA therapy. A CPs of 9 or more represents a major risk factor for the aggravation of liver function after RFA therapy.

Key words: Child–Pugh score, hepatocellular carcinoma, liver cirrhosis, liver function parameters, percutaneous radiofrequency ablation therapy

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most significant malignancies among chronic liver diseases (CLD).[1] Surgical treatment such as hepatectomy is curative for HCC if the tumor is localized to the liver.[2] However, this therapy is frequently contraindicated by concomitant liver disease, particularly cirrhosis with severe liver damage. Furthermore, although liver transplantation remains the ultimate therapy for patients who fulfill the Milan criteria for liver transplantation and obtain a donor liver, this situation remains limited.[3]

Percutaneous radiofrequency ablation (RFA) therapy has recently been developed as an effective therapy for HCC patients who do not undergo surgery.[4,5] However, as hepatitis B virus (HBV) and hepatitis C virus (HCV) are major causes of HCC in Japan, the recurrence rate of HCC after initial treatment is particularly high.[6,7] If patients with HBV- or HCV-related hepatitis cannot receive antiviral therapies and surgical treatment, repeated therapy for recurrence of HCC is required, resulting in deterioration of impaired liver function. Maintaining residual liver function capacity is thus very important to appropriately treat recurrent HCC. When HCC therapy is performed, changes to liver function are inevitable.[8] This phenomenon is not specific for RFA.

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However, precisely how residual liver function in HCC patients changes in the long term after RFA therapy remains unclear.

The goal is early detection and appropriate treatment of HCC in addition to stable maintenance of liver function to prolong the survival time of patients. The present study examined changes in liver function parameters according to Child–Pugh (CP) classification after RFA therapy and clarified risk factors for aggravation of liver function.

METHODS

Subjects

AMONG 55 PATIENTS with HCC who had undergone RFA therapy at the Division of Gastroenterology and Hepatology, Department of Internal Medicine, Iwate Medical University Hospital between January 2001 and March 2003, subjects comprised 45 patients who showed no HCC recurrence (local and/or intrahepatic) at 1 year after RFA therapy.

Patient profiles at the time of initial RFA therapy for HCC are shown in Table 1. During follow up, no patients received any other therapies, such as administration of albumin (Alb) infusion or antiviral treatments that might influence liver function. All patients with HCC showed CLD, comprising chronic hepatitis (CH) in eight patients and liver cirrhosis (LC) in 37 patients. CH and LC were diagnosed by biochemical examination of markers of liver fibrosis, imaging methods using endoscopy, ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI), and liver biopsy. Etiologies of hepatitis were: HBV, positive hepatitis B surface antigen; HCV, positive anti-hepatitis C antibody and/or HCV RNA; alcohol, more than 80 g/day ethanol consumption over 10 years; or unknown, no HBV, HCV or alcohol intake. Peripheral blood counts (red blood cells, white blood cells and platelet counts), liver function tests, prothrombin time (PT) and tumor markers in the blood (α-fetoprotein [AFP], lectin 3 fraction of AFP [AFP-L3] and protein induced by vitamin K absence or antagonist II [PIVKA-II]) were measured using commercial kits. Diagnosis of HCC and the number of nodules were detected by abdominal US, dynamic CT and/or MRI. Liver biopsy was performed in 12 patients to confirm the diagnosis of HCC.

Methods

The Cool-tip Radiofrequency System (Radionics, Burlington, MA, USA) has been used for RFA therapy of HCC in our institute since 2000. Indications for RFA were: (i) unresectable HCC or refusal of surgery; (ii) absence of uncontrollable ascites and hepatic encephalopathy; or (iii) absence of marked bleeding tendency. The general indications are patients with three lesions or less, all of which are 3 cm or less in diameter, or a single lesion of 5 cm or less. Twenty patients underwent transcatheter arterial embolization (TAE) prior to RFA therapy. For these patients, the mean interval from TAE to starting RFA therapy was 16.5 days (range, 8–58 days). RFA therapy was performed once or twice a week until complete necrosis of HCC was confirmed on dynamic CT.[9] RFA therapy was performed a mean of 1.8 ± 0.7 times in the study subjects.

Serum total bilirubin (T-Bil), Alb and PT were evaluated and CP score (CPs) was determined before and 3, 6

Table 1 Patients profiles at the time of initial treatment for hepatocellular carcinoma

Parameters	$n = 45$
Sex (male/female)	27/18
Age (years)	69 (66.2 ± 9.1)
Hepatitis B/C/NBNC/alcohol	3/37/3/2
Liver disease (CH/LC)	8/37
Child–Pugh grade (A/B/C)	19/16/2
Child–Pugh score (5/6/7/8/9/10 points)	11/8/9/3/4/2
Stage (I/II/III)	14/18/13
Number of tumor(s)	63
Solitary/multiple	24/21
Size of tumor (mm)	22 (25.2 ± 8)
Biological parameters	
T-Bil (mg/dL)	0.9 (0.9 ± 0.4)
Alb (g/dL)	3.6 (3.6 ± 0.4)
ALT (IU/L)	39 (41 ± 18)
Plt ($\times 10^4$/mm^3)	10.4 (11.2 ± 3.5)
PT (%)	76 (72 ± 14)
Tumor markers	
AFP (>100 ng/mL, positive/negative)	19/27
AFP-L3 (>10%, positive/negative)	10/35
PIVKA-II (>40 mAU/mL, positive/negative)	20/25
Treatment (with/without TAE)	20/25
BCAA administration (yes/no)	13/32
Liver volume (cm^3)	988.3 ± 245
Ablated volume (cm^3)	31.5 ± 25.4
Ablated rate (%)	3.2 ± 2.9

AFP, α-fetoprotein; AFP-L3, lectin 3 fraction of AFP; Alb, aluminum; ALT, alanine transaminase; BCAA, branched-chain amino acid; NBNC, non-B non-C; Plt, platelet counts; PIVKA-II, protein-induced vitamin K absence or antagonist II; PT, prothrombin time; TAE, transcatheter arterial embolization; T-Bil, total bilirubin.

and 12 months after RFA therapy.[10] Volumetric analysis of the entire liver and ablated portion was performed as follows: the area of liver parenchyma and ablated area were calculated using image-processing software attached to the CT system.[11,12] Parenchymal ablation rate was calculated as: ablated volume/liver parenchymal volume × 100 (%).[13] The relationship between change rate of CPs (ΔCPs) and grading of CP classification was evaluated. Aggravation of liver function was defined when CPs was increased by 2 or more at 12 months after RFA therapy. In addition, Cox proportional hazards regression analysis was performed to clarify risk factors for aggravation of liver function after RFA therapy.

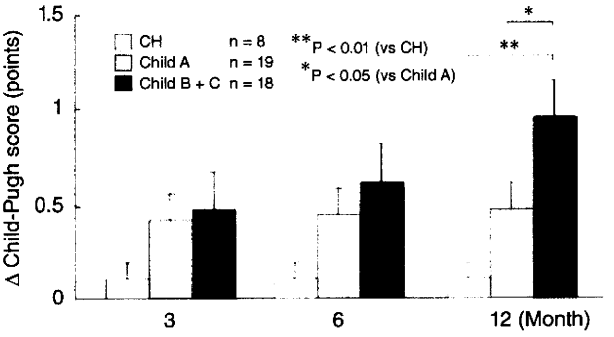

Figure 1 The changes of Δ Child–Pugh score after radiofrequency ablation.

Statistics

Laboratory data are shown as mean ± standard deviation. Statistical analyses were performed using StatView ver. 5.0 software. Cox proportional hazards regression was performed to evaluate risk factors for aggravation of liver function using demographic data obtained prior to initial RFA. Univariate analysis evaluated 20 factors: age; sex; HCV; T-Bil; alanine aminotransferase; Alb; platelet count (Plt); PT; AFP; AFP-L3; PIVKA-II; grading of CP classification; CPs; tumor size; number of tumor nodules; combination therapy with TAE; branched chain amino acids (BCAA) treatment; ablated rate; recurrence of HCC; and complications. Parameters identified as significant in univariate analysis were tested in the multivariate Cox proportional hazards model for all patients.

RESULTS

Serial changes in CPs after RFA therapy

SERUM ALB LEVELS decreased significantly after RFA therapy from 3.6 ± 0.4 g/dL before RFA to 3.5 ± 0.3 g/dL after 6 months and 3.2 ± 0.4 g/dL after 12 months ($P \leq 0.05$). Serum T-Bil and PT levels were unchanged after RFA (T-Bil: before, 0.9 ± 0.4 mg/dL; after 12 months, 1.0 ± 0.4 mg/dL; PT: before, 76.1 ± 14.5%; after 12 months, 74.8 ± 15.2%). CPs was significantly increased after RFA from 6.4 ± 1.4 before RFA to 6.8 ± 1.9 after 6 months ($P \leq 0.05$) and 6.9 ± 2.0 after 12 months ($P \leq 0.01$). Serial changes in ΔCPs according to grading of CP are shown in Figure 1. No change in ΔCPs was seen in CH or LC patients with grade A at 12 months after RFA therapy, while significant increases were noted in grade B and C LC patients. Changes in Alb, T-Bil, and CPs did not differ between patients receiving RFA alone and those receiving TAE ± RFA (data not shown).

Risk factors for aggravation of liver function

Univariate analysis showed CPs (≥9), Plt and Alb as predictive factors for deterioration of liver function after RFA therapy, while CPs (≥9) was the only significant risk factor identified by stepwise multivariate analysis (Table 2). In addition, no significant differences were observed between patients with and without TAE.

DISCUSSION

THE PRESENT STUDY evaluated serial changes in T-Bil, Alb and PT, three parameters used in the CP classification, over the course of 12 months following RFA therapy. We also tried to identify risk factors for long-term aggravation of liver function after RFA. Our data indicated that: (i) Alb is significantly decreased 12 months after RFA; (ii) CPs is significantly increased both 6 and 12 months after RFA; and (iii) CPs in patients with CH and grade A LC does not change after RFA. Furthermore, multivariate analysis identified CPs of 9 or more as a major risk factor for the aggravation of liver function following RFA. Taken together, these data suggest that appropriate nutritional support is necessary for HCC patients over grade B and C or with a CPs of 9 or more when RFA therapy is performed.

However, the study population comprised patients receiving two therapies, namely, patients treated with RFA therapy alone and patients treated with TAE prior to RFA therapy. We therefore did not confirm a direct influence of RFA therapy alone, although liver function tests before RFA therapy showed similar findings in both groups and the performance of TAE was not identified as a significant risk factor for the aggravation of liver func-

Table 2 Risk factors contributing to degradation of functional reserve of the liver after radiofrequency ablation

Univariate analysis		
Variables	Odds ratio (95% CI)	*P*
Age (>69 years)	2.18 (0.45–10.53)	0.329
Sex (male)	5.95 (0.61–53.3)	0.111
HCV antibody (positive)	0.58 (0.94–3.59)	0.559
Size of tumor (>22 mm)	0.45 (0.95–2.22)	0.329
Number of tumor (multiple)	1.44 (0.25–8.23)	0.682
Child classification (Child B/C vs Child A/CH)	3.07 (0.79–14.87)	0.163
Child-Pugh score (≥9 points)	16.5 (2.39–127.53)	0.004
T-Bil. (>0.9 mg/dL)	1.57 (0.32–7.59)	0.124
Alb (<3.5 g/dL)	5.18 (1.02–26.43)	0.047
ALT (>80 IU/l)	1.92 (0.72–3.53)	0.694
Plt (<9.4 × 10^4/mm^3)	7.91 (1.23–40.75)	0.028
PT (<70%)	3.16 (0.52–17.53)	0.194
AFP (>100 ng/mL)	0.87 (0.19–4.52)	0.873
AFP-L3 (>10%)	1.55 (0.19–8.53)	0.685
PIVKA-II (>40 mAU/mL)	0.81 (0.18–3.23)	0.835
Treatment (with/without TAE)	0.76 (1.47–3.39)	0.668
BCAA administration (yes)	2.78 (0.88–13.53)	0.148
Ablated rate (>3.5%)	2.35 (0.15–12.53)	0.651
Recurrence (+ vs –)	3.10 (0.57–16.58)	0.168
Complication (+ vs –)	2.54 (0.19–31.55)	0.483
Stepwise multivariate analysis		
Variables	Odds ratio (95% CI)	*P*
Child-Pugh score (≥9 points)	8.02 (1.05–5.67)	0.024
Plt (<9.8 × 10^4/mm^3)	3.52 (0.59–1.24)	0.347
Alb (<3.5 g/dL)	2.52 (0.94–1.14)	0.401

Alb, aluminum; AFP, α-fetoprotein; AFP-L3, lectin 3 fraction of AFP; ALT, alanine aminotransferase; BCAA, branched-chain amino acid; CI, confidence interval; HCV, hepatitis C virus; PIVKA-II, protein-induced vitamin K absence or antagonist II; Plt, platelet counts; PT, prothrombin time; TAE, transcatheter arterial embolization; T-Bil, total bilirubin.

tion after RFA therapy. Because no studies have yet compared changes in liver function tests between RFA therapy and TAE therapy, the differences between these therapies need to be clarified in the future.

Patients with advanced LC and malnutrition are well known to exhibit poor prognosis.[14] Previous studies have shown that p.o. administration of BCAA can improve nutritional status and event-free survival in cirrhosis.[15] Recent studies have also suggested potential benefits of BCAA administration in cirrhotic patients with HCC.[16–19] The present study, however, could not convincingly examine the effects of BCAA in HCC patients receiving RFA, because the number of patients receiving BCAA was small (13 of 45 patients, grade A, $n = 4$; grade B or C, $n = 9$) and doses and formulations of BCAA varied among patients. Further studies are necessary to fully evaluate the effects of BCAA on nutritional status and recurrence of HCC in patients with LC following RFA.

In addition, as shown in the results, BCAA treatment was not a significant risk factor for deterioration of liver function after RFA therapy in univariate analysis. The following reasons are considered to explain why BCAA treatment in this study did not improve serum Alb level. First, the number of patients receiving BCAA treatment was very small. Second, administered doses and formulations of BCAA (e.g. BCAA granules and BCAA-enriched nutrients) were not fixed. In the future, differences between BCAA granules and BCAA-enriched nutrients should be evaluated with regard to influences on nutritional status and recurrence of HCC in LC patients. The present results might be useful as baseline data to estimate the effects of nutritional support therapies among HCC patients receiving RFA therapy.

In conclusion, serum Alb level gradually decreases over the course of 1 year after RFA therapy in LC patients with grade B or C according to the CP classification. A CPs of 9 represents a critical baseline to estimate progression to liver failure in HCC patients after receiving RFA therapy.

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今月のテーマ●肝硬変の包括的マネージメント

肝性脳症治療の up-date

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要旨：肝硬変に起因する肝性脳症の病態には肝細胞障害と門脈大循環短絡の２つの要因が相互に関連している．肝性脳症は精神神経障害の程度により昏睡Ⅰ度からⅤ度までに分類されるが，最近，明らかな精神神経症状がなく定量的精神神経機能検査ではじめて異常を指摘される潜在性（ミニマム）肝性脳症の病態が注目されている．しかし，その診断法についてのコンセンサスはいまだ得られていない．治療では血液アンモニア濃度のコントロール，アミノ酸代謝異常の是正を目標に合成二糖類，難吸収性抗菌薬，特殊組成アミノ酸製剤（分岐鎖アミノ酸製剤）などが用いられているが，その効果は肝の重症度に大きく影響される．肝不全が高度の例に対しては血液浄化療法，肝移植も行われている．蛋白・エネルギー代謝異常の是正が肝性脳症の顕性化予防，長期予後の改善には重要な対策と考えられる．

索引用語：肝硬変，肝性脳症，合成二糖類，難吸収性抗菌薬，分岐鎖アミノ酸療法

はじめに

肝性脳症（肝性昏睡）は肝硬変（肝癌合併例も含む）の経過中にみられる重篤な合併症の１つである．肝性脳症の発生機序には，アンモニアを中心とした中毒性物質による多因子説，アミノ酸代謝異常説，偽性神経伝達物質説，γアミノ酪酸(GABA)/ベンゾジアゼピン受容体複合体異常説などがあるが，単一の機序では説明が困難である．近年，画像診断の進歩により，門脈血行動態における門脈大循環短絡の多様性が明らかとなり，また，脳における神経伝達物質および種々の代謝物質の動態などが検討され，肝性脳症の病態解析が進んでいる．さらに，潜在性肝性脳症（意識状態が一見正常と判断される例において定量的精神神経機能検査を行うと少なからず異常を認める例）の概念が提唱され，その診断，病態，臨床的意義について議論されてきている．一方，治療では分岐鎖アミノ酸（BCAA）製剤の位置づけが明確にされ，肝性脳症の改善のみならず蛋白アミノ酸代謝異常の是正やQOLの改善，さらには発癌抑制の可能性も示唆されてきており，新たな展開を迎えている．しかし，高度の肝細胞機能障害をともなう肝硬変例では，今なお治療に難渋することが多く，予後も不良であり，最終的には肝移植を受ける例が増えつつある．

本稿では，とくに肝硬変に起因する肝性脳症の治療について最新の知見を含めて解説する．

Ⅰ　臨床病型

最近，欧米では，新しい肝性脳症の分類(Table 1）が提案されており，肝硬変による肝性脳症はC型として，さらに，エピソード型，持続型，ミニマル肝性脳症（潜在性肝性脳症）に分類している[1)]．この分類では肝性脳症の発症様式や持続期間を重視しており，肝疾患とは異なる原因によって生じる大脳疾患を除外しているが，アンモニア血症をきたす先天性尿素サイクル異常症（高シト

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Table 1. 肝性脳症の新しい分類

型	名称	サブカテゴリー
A（Acute）型	急性肝不全（劇症肝炎など）でみられる脳症	
B（Bypass）型	門脈～大循環系バイパスによる脳症で，肝硬変などの肝疾患をともなわない	
C（Cirrhosis）型	肝硬変と門脈圧亢進症/門脈～大循環短絡路バイパスでみられる脳症	
	エピソード（間欠）型脳症	1. 誘因あり型 2. 誘因なし型 ①再発型（2回以上/年） ②非再発（特発）型
	持続型脳症	1. 軽症型（grade I） 2. 重症型（grade II～IV）
	ミニマル脳症	3. 治療依存型 潜在性脳症といわれたもの

Table 2. 肝硬変による肝性脳症の臨床病型

1. シャント型：
   門脈—大循環短絡（portal-systemic shunt）によりアンモニアなどの中毒性物質が門脈より直接大循環に流入することによる．多くの肝硬変（肝癌合併例も含む），特発性門脈圧亢進症などが該当し，明らかな誘因を認める例が多い．
2. 肝細胞障害型：
   末期昏睡型とも呼ばれる．門脈—大循環短絡を伴うが肝細胞障害因子が強い例．肝硬変のうち高度の黄疸や肝機能異常を伴う例が該当する．誘因不明例が多い．

ルリン血症など）による肝性脳症例の位置づけが明確でなく，サブカテゴリーについてもさらに検討が必要と考えられる．ミニマル肝性脳症は潜在性肝性脳症[2]と同一の病態であり昏睡度ゼロの状態を意味するが，この病態を顕性脳症の前段階の病態と捉えるか否かについてはいまだ議論が分かれている．また，潜在性肝性脳症は知識，数唱，単語といった言語性の認知能は比較的保たれるのに対して，動作性の認知能の低下が特徴とされる．したがって，WAIS知能検査のうち積木試験（block design test），符号試験（digit symbol test），さらには数字追跡試験（number connection test A and B）の3項目を実施し，どれか1項目に異常を認める場合に潜在性肝性脳症と診断することが多い．しかし，診断のための検査法としてのゴールドスタンダードが確立されていないため，その実態（頻度，予後）は報告者によって異なっている[3)~6)]．最近，わが国でもコンピューターを用いた精神神経機能検査法が開発され，罹病率や脳症の顕性化率などが検討されている[7]．

一方，わが国では肝硬変による肝性脳症を肝細胞障害の強いタイプ（肝細胞障害型あるいは末期昏睡型）と門脈大循環シャント因子が強いタイプ（シャント型あるいは慢性再発型）に分類している（Table 2）．この分類は，肝硬変の病態には肝細胞機能障害と門脈大循環シャントの2つの要因が相互に関連していることを基本とし，治療の反応性や予後を重視したものである[8)9)]．しかし，ミニマル肝性脳症などの位置づけは明確にされておらず，今後，欧米との整合性を図る必要があると考えられる．肝細胞障害型とシャント型における肝病態の特徴として，前者では高度の黄疸と肝予備能の著しい低下を認めるが，一方，後者では比較的肝機能は良好である．アンモニア処理能の低下，尿素合成能の低下および門脈大循環シャントによる血液アンモニア濃度の上昇，Fischer比（BCAA/AAA）の低下あるいはBCAA/Tyr（チロシン）比の低下は両病型とも共通してみられ

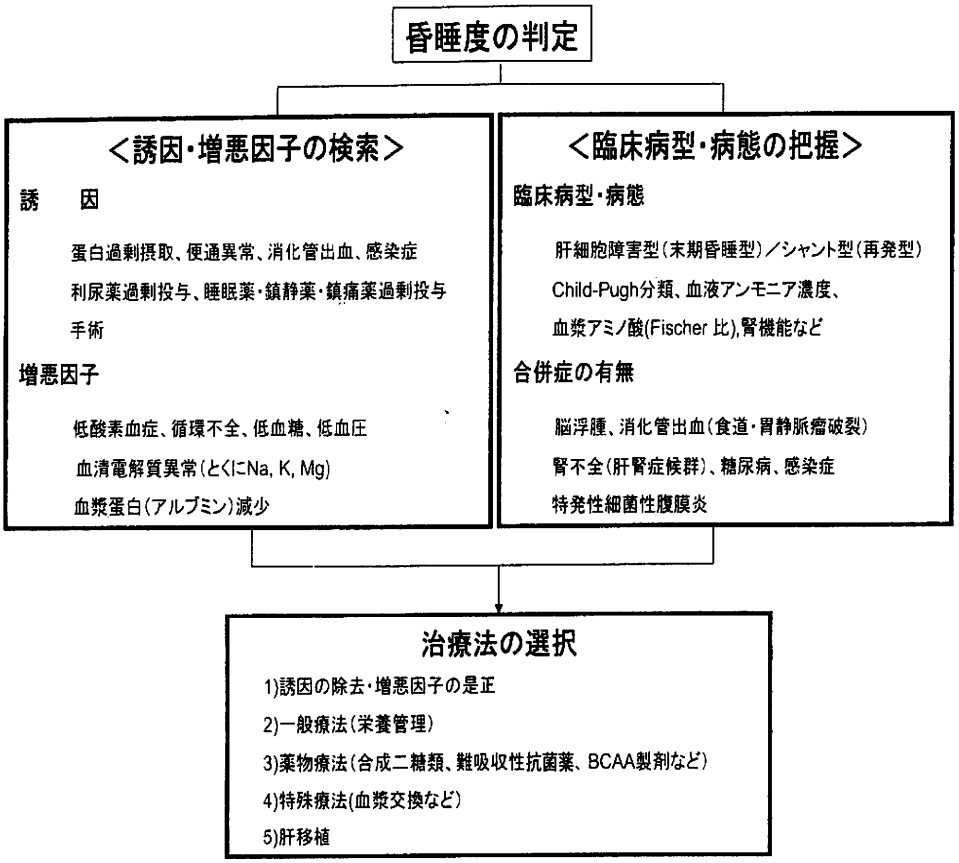

Figure 1. 肝硬変肝性脳症の治療指針

る．なお，先天性尿素サイクル代謝異常症では血液アンモニア濃度の著明な上昇がみられ，それぞれの疾患に応じた特徴的なアミノ酸の変動が観察される．

肝性脳症の重症度は通常昏睡度で表す．わが国では犬山シンポジウムによる昏睡度分類（I～V度）が広く用いられている．欧米ではWest Havenクライテリアとして4段階に分類することが提唱されているが[2]，いずれにしても昏睡I度の判定が難しく，retrospectiveにしか判定できない場合が多い．欧米の分類では昏睡I度の判定としてTMT-Aなどによる評価を提唱している[3]．

II 治 療

肝性脳症の誘因や増悪因子，臨床病型および合併症の有無を把握して，治療方針を決定する(Figure 1)．代表的な誘因として食事蛋白量の過剰摂取，消化管出血，便通異常（とくに便秘），感染症，鎮静剤・鎮痛剤の過剰投与，利尿剤の過剰投与による電解質異常・脱水などがあり，われわれの成績[10]では約70%の例に何らかの誘因を認める．近年は消化管出血による肝性脳症例が減少し，誘因不明例が増加している．肝癌合併例では腹腔内出血も肝性脳症の誘因の1つとなる．また，脳症の増悪因子として低酸素血症，循環不全，低血糖，低血圧，血清電解質異常（とくにナトリウム，カリウム，マグネシウム），血漿蛋白（アルブミン）減少などがある．

治療では，誘因の除去・増悪因子の是正とともに，腸管内の清浄化（アンモニアなどの中毒性物質の産生および吸収を抑制する，腸内細菌によるアンモニアの産生を抑制）を図る対策が基本であり[11]，これに肝細胞機能の改善を図る対策が加わる．肝硬変による肝性脳症の場合，たとえ昏睡IV度またはV度であってもシャント型では短期的には完全意識覚醒が得られることは可能である．しかし，いずれの病型であっても内科的治療による生命予後は必ずしも満足すべきものではない[12]．

Table 3. ESPEN ガイドライン（2006）

●一般的事項
・SGA や身体計測により低栄養状態のスクリーニング
・推奨摂取熱量：35 ～ 40kcal/kg/日
・推奨摂取蛋白質量：1.2 ～ 1.5g/kg/日
●経腸栄養の適応
・適切な栄養指導を行っても必要量を経口的に摂取できない場合
●経路
・食事が至適量に満たない場合，経腸栄養剤を経口 or 経管投与
●経腸栄養製剤の組成
・一般的な蛋白組成が推奨される
・腹水症例では高蛋白・高カロリーの組成を考慮
・肝性脳症を発症した例では BCAA 高含有製剤を投与
・経口的 BCAA 補充は肝硬変の予後を改善
●予後
・経腸栄養療法は栄養状態，肝機能を改善，合併症を減らし，生存期間を延長

なお，ミニマル肝性脳症例の経過観察を行うと，少なからず脳症の顕性化が認められることより[13]，血液アンモニア濃度が高値傾向を示す例では治療の対象になる．

1．一般的治療（全身管理）

栄養管理では昏睡 III 度以上または経口摂取不能時（食道静脈瘤破裂など）には絶食とし糖質を中心とした静脈栄養管理を行う．アミノ酸製剤は BCAA を多く含有し AAA を少なく配合した特殊組成アミノ酸製剤を用いる．ただし，肝細胞障害型では投与されたアミノ酸が過剰な窒素負荷となり，むしろ肝性脳症を悪化させる可能性があるので投与量・投与法には注意が必要である．昏睡 II 度以内となり経口摂取が可能となった場合には，通常，食事蛋白量を制限（蛋白量 1 日 40g 以下）し，これに BCAA を多く含有する経腸栄養剤を併用投与する[14]．長期間の蛋白制限食は蛋白の異化亢進を助長し蛋白・エネルギー栄養障害（protein-energy malnutrition；PEM）をさらに悪化させるので，経腸栄養剤の併用は必須である．最近，急性エピソード型の肝性脳症例に対する食事蛋白量についての報告[15]があり，通常の蛋白量でも肝性脳症の悪化は認められないという．しかし，この研究では同時に抗菌薬（ネオマイシン）の投与が行われており，また短期間（2 週間）の観察であることより今後の追試が必要である．

また，糖尿病を合併する例では総投与熱量を制限し，必要に応じて血糖降下薬やインスリンを投与するが，Late evening snack（LES）を行うことにより耐糖能の改善が得られる可能性があることが報告されている[16]．

Table 3に肝硬変に対する栄養療法のガイドライン（ESPEN）を示す[15]．最近は欧米でも BCAA の重要性を考慮してきている[17]．

2．薬物療法

1）合成二糖類（ラクツロース，ラクチトール）

肝性脳症治療に用いる基本的な薬剤である．ミニマル肝性脳症にも有効性が示されている[18][19]．臨床効果（昏睡改善作用，血液アンモニア濃度の低下作用）は両剤とも同程度であるが，ラクツロースは甘味が強く高容量の長期服用においてはコンプライアンスの面で問題がある．副作用（悪心，嘔吐，腹痛，腹部膨満感，下痢など）の発現頻度はラクチトールがラクツロースよりも少ない．

投与方法は，経口（時に経管的）投与と経腸投与（浣腸）がある．ラクツロース（シロップ製剤）は便の性状と排便回数（1 日 2～3 回）を目安に 1 日 30～90m*l* を 3～4 回に分けて使用する．ラクチトール（粉末製剤）は水に溶解して 1 日 18～36 g を使用する．緊急時には経口投与量の 3～10 倍量を水または生理的食塩水で希釈し高圧浣腸を行う．われわれの施設ではラクツロース 100m*l* を

微温湯または生理的食塩水100mlに混じて1日1～2回浣腸する方法を施行している．また，ラクツロース300mlに微温湯または生理的食塩水700mlを混じて注腸して1時間後に排液させ，これを4～6時間ごとに繰り返す方法もある．

2）難吸収性抗菌薬

合成二糖類による治療で高アンモニア血症の改善が得られない場合に使用するのが一般的である．基本的には腸管より吸収されない抗菌薬を使用する．硫酸カナマイシン，硫酸フラジオマイシン（ネオマイシン）を1日2～4g，分2～3で経口投与する．抗エンドトキシン作用を有する硫酸ポリミキシンB（1日300～600万単位，分3）やアンモニア産生能の高い嫌気性グラム陰性桿菌を特異的に抑制する塩酸バンコマイシン（1日2g，分2～3）の有効性も報告されている．これらの抗菌薬は保険診療適応外の薬剤であるが，血液アンモニア濃度のコントロールが不十分な例，肝性脳症を繰り返す例などでは試みる価値はある．また，欧米では難吸収性抗菌薬であるRifaximin（オーファンドラックで広域スペクロラムを有する抗菌薬，1.2g/日，5～10日間投与，最近，米国FDAでは旅行者の下痢に対する使用に許可された）の有効性を示す成績が多く報告され，ラクツロースに比し効果が高い[20][21]．また，血清ベンゾジアゼピン様物質濃度は，本剤投与時のみ減少し，ラクツロースでは不変との報告[22]もある．したがって，難吸収性抗菌薬の投与は腸管内で発生するアンモニアなどの中毒性物質の産生を抑える意味では理にかなっており，ラクツロースよりも脳症改善効果が期待される．しかし，長期使用の安全性は確立していない．

3）特殊組成アミノ酸製剤

BCAA高含有の輸液製剤と経腸栄養剤，およびBCAA顆粒製剤があり，臨床病期（昏睡極期，回復期または意識覚醒期）やPEMの有無によって使い分ける．輸液製剤は昏睡期に使用するが，血中および脳内のアミノ酸インバランスの是正による脳内神経伝達障害の改善を図ることを目的に開発された製剤であり，シャント型に対しては極めて速効性の効果を示す．これに対して，肝細胞障害型では肝の重症度が増すほど改善効果は低率であり，一過性に終わることも少なくない．特殊組成アミノ酸輸液製剤を用いた治療法による意識覚醒効果は従来の治療法（ラクツロース・抗菌薬）に比べて完全覚醒までの日数が有意に短い[23]．

経腸栄養剤は前述したように，昏睡II度以下あるいは経口摂取が可能で腸管の運動機能異常のない例が適応となる．本剤は当初は肝性脳症の改善を目的に開発されたが，その後，血中のアミノ酸インバランスの是正のみならず肝硬変にみられるPEMに対する治療法として確立してきている．LESとしてアミノレバンENを用いた比較試験成績[24]では，低アルブミン血症，貧血などの改善とともに，脂肪・炭水化物の燃焼比率および非蛋白呼吸商（npRQ）の改善を認めている．最近，米国でもLESの有用性に関する報告もなされた[25]．軽食によるLESの効果も報告されているが，長期間にわたって行うことを考慮するとBCAAを多く含有する経腸栄養剤はより効果的である．

BCAA顆粒（配合比ほぼ1.2：2：1）は低アルブミン血症に代表される低栄養状態の是正を目的に開発された製剤である．通常，1日3包（BCAA 12g）を食後3回に分けて服用するが，LES時の併用も有用との報告もある．最近，低栄養状態をともなう肝硬変に対するBCAA顆粒の長期投与の有用性が報告され，BCAA顆粒投与群では食事療法群に比較してイベント発生率（経過中の腹水，浮腫，肝性脳症，黄疸などの増悪，食道・胃静脈瘤破裂，肝癌合併，他の原因による死亡）が有意に低いことが証明された[26]．以上の成績により，肝性脳症例を含む低栄養状態をともなう肝硬変患者のQOLを維持するためにはBCAAの補充やLESによる栄養療法が基本的な治療法となりつつある．

4）その他

アンモニア代謝を是正する目的で，亜鉛製剤（酢酸亜鉛 1日 600mg，硫酸亜鉛 1日 300mg，ともに経口投与），安息香酸ナトリウム（急性期 250mg/kgを90分以上かけて点滴静注，血液アンモニア濃度の低下を確認後 250mg/kg/日で持続点

滴，経口投与では250mg以内/kg/日）が使用されている．後者は主として先天性尿素サイクル異常症に使用される．

ベンゾジアゼピン受容体拮抗薬（フルマゼニル）による治療も試みられている[27]．肝性脳症に対する意識覚醒効果は限定されるが，わが国ではアルコール性の肝硬変では有効との成績が報告されている．

α-グルコシダーゼ阻害薬であるアカルボースが2型の糖尿病を合併しかつ昏睡II度以内の肝硬変肝性脳症の治療薬として有効との報告[28]もみられる．また，α-グルコシダーゼ阻害薬はLESが困難な例に対するエネルギー代謝異常の是正することが示されており，今後の展開が期待される薬剤と考えられる[29]．

最近，ミニマル肝性脳症例に対するプロバイオティクス製剤（1カプセル中に*Streptococcus faecalis* 60億菌数，*Clostridium butyricum* 4億菌数，*Bacillus mesentricus* 2億菌数含有）の有用性に関する報告[30]がなされた．この報告ではラクツロース単独群（30～60ml/日），プロバイオティクス製剤単独群（3カプセル/日）および両者の併用群の3群に分けて1カ月観察しているが，いずれの群でも精神神経機能，事象関連電位（P300）および血液アンモニア濃度の改善がみられている．

この他，不眠を訴えるミニマム肝性脳症例に対するヒスタミンH1ブロッカーの有効性に関する無作為比較試験の報告がなされている[31]．また，実験的にはIbuplofen（消炎鎮痛薬）[32]，Sildenafil（phosphodiesterase阻害薬）[33]の効果が報告されている．

3. 人工肝補助療法

血漿交換と持続血液濾過透析を組み合わせた人工肝補助療法は主に肝細胞障害型や高シトルリン血症などの先天性尿素サイクル異常症による肝性脳症例に対して行われている．しかし，その効果は一過性である．一方，欧米ではアルブミン透析であるMARS（molecular adsorbents recirculating system）が主流であり，肝性脳症やアミノ酸プロファイルの改善，アルブミン結合能の改善などに有効とされる[34)~36)]が，あくまでも肝移植までのbridge useとしての位置づけにある．

III 肝移植

2004年に肝移植対象疾患の保険拡大がなされたことよりB型およびC型肝硬変例や肝癌例に対する移植例が増加してきている．しかし，わが国では大部分が生体部分肝移植であるため，ドナー肝が得られなければおのずとその予後は極めて不良である．

肝硬変での肝移植適応は末期肝不全状態を示す例であるが，その判定はMELDスコアによるのが一般的である（15点以上）[37]．一方，肝癌合併例ではミラノ基準[38]が用いられる．

肝性脳症を繰り返していた肝硬変による移植例では，脳内の代謝機能が改善することが報告されているが，脳症の進んだ例では移植を行っても完全に神経症状が改善しない例も報告されている．さらに，肝炎ウイルスが成因の移植例では移植後も抗ウイルス療法が必須であるため，移植後も厳重な経過観察が必要である．

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Alterations in expression of genes coding for proteins of the neurovascular unit in ischemic liver failure

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ABSTRACT

There is evidence to suggest that integrity of the neurovascular unit may be compromised in acute liver failure (ALF). In order to address this issue from a molecular standpoint, expression of an array of genes coding for key cerebrovascular endothelial cell and tight junction proteins were measured by reverse transcription-polymerase chain reaction in cerebral cortex of rats with ischemic liver failure resulting from hepatic devascularization (portacaval anastomosis followed 24 h later by hepatic artery ligation) compared to appropriate sham-operated controls. Expression of P-glycoprotein, endothelin-1, von Willebrand factor, caveolin-1, occludin, and the endothelial nitric oxide synthase isoform (eNOS) were measured in brain extracts from rats with ALF at coma/edema stages of encephalopathy. The effects of mild hypothermia (35 °C) sufficient to prevent cerebral edema in ALF animals on the expression of these genes were also studied. Brain edema and hepatic coma in normothermic ALF rats was accompanied by selective increases in expression of eNOS. Expression of occludin and von Willebrand factor mRNAs were decreased at coma/edema stages of encephalopathy in ALF rats whereas, expression of other cerebrovascular endothelial cell markers endothelin-1, P-glycoprotein, and caveolin-1 were unaffected. Mild hypothermia led to normalization of brain water content and of eNOS mRNA. However, the correlation between increased eNOS expression and encephalopathy/edema grade was poor suggesting the existence of additional mechanisms. These findings underscore the multifactorial nature of brain edema/encephalopathy mechanisms in ALF and question the role of BBB breakdown as a major pathogenetic factor.

1. Introduction

Hepatic encephalopathy (HE) and brain edema leading to intracranial hypertension and brain herniation are serious neurological complications of acute liver failure (ALF). The precise pathophysiologic mechanisms responsible for these complications are not completely understood. One possible mechanism involves alterations of the neurovascular unit and blood–brain barrier (BBB) dysfunction. Histopathological studies reveal discreet alterations of cerebrovascular endothelial cells (Kato et al., 1992; Potvin et al., 1984) as well as altered expression of genes coding for BBB proteins (Shimojima et al., 2008). Moreover, ammonia, a neurotoxin known to accumulate in brain to millimolar concentrations in ALF (Swain et al., 1992) has been shown to cause decreased expression of the tight junction (TJ) protein claudin-12 (Bélanger et al., 2007) and, under certain conditions, ammonia exposure leads to increases in effective pore size of the BBB (McClung et al., 1990).

As part of a series of studies to assess the neurovascular unit in relation to the pathogenesis of HE and brain edema in ALF, expression of an array of genes coding for some key BBB proteins were measured by reverse transcription-polymerase chain reaction in cerebral cortex of rats with ALF resulting from hepatic devascularization compared to sham-operated controls. Expression of the TJ protein occludin and von Willebrand factor (vWF), caveolin-1 (Cav1), P-glycoprotein (P-gp), endothelin-1 (ET-1) and the endothelial isoform of nitric oxide synthase (eNOS) mRNAs were measured in groups of animals maintained at 37 °C compared to a similar group maintained mildly hypothermic (35 °C) sufficient to prevent signs of encephalopathy and to prevent brain edema in these animals (Rose et al., 2000; Stravitz et al., 2008).

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Abbreviations: HE, hepatic encephalopathy; HAL, hepatic artery ligation; ALF, acute liver failure; BBB, blood–brain barrier; ET-1, endothelin-1; eNOS, endothelial nitric oxide synthase; P-gp, P-glycoprotein; vWF, von Willebrand factor; Occ, occludin; Cav1, caveolin-1.

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2. Materials and methods

2.1. Surgical procedures

Adult male Sprague–Dawley rats (200–250 g) purchased from Charles River (Saint-Constant, Quebec, Canada) were routinely tested for common pathogens and were free of infection at the onset of surgery. Rats were anesthetized with isoflurane, and an end-to-side portacaval anastomosis was performed according to the guidelines of Lee and Fisher (1961). Briefly, rats underwent a laparotomy, the inferior vena cava and portal vein were isolated and clamped using an anastomosis clamp (Roboz Instruments Inc., Washington, DC) and an elliptical portion 1.5 times the diameter of the portal vein was removed. The portal vein was ligated and cut, and an end-to-side anastomosis was performed under a dissecting microscope. Total surgery time was <30 min. Sham-operated control rats, matched for weight, were similarly anesthetized and the inferior vena cava was clamped for 20 min. Following surgery all animals were individually housed with free access to food and water under constant conditions of temperature, humidity and light cycles. Twenty-four hours after portacaval anastomosis, rats were reanesthetized and subjected to hepatic artery ligation (HAL). Following HAL, arterial blood glucose levels were monitored and glucose was administered subcutaneously as needed to maintain normoglycemia. Body temperature and reflexes were monitored every 15 min and temperature was maintained at 37 °C ± 0.5 °C by means of heating pads. Hypothermia occurred spontaneously in the absence of external heating and body temperature was maintained at 35 °C ± 0.5 °C using heating pads when necessary. A group of animals was sacrificed 6 h (prior to the appearance of encephalopathy and brain edema) following HAL (ALF-6 h). A second group was sacrificed approximately 13 h following HAL (ALF-37) at the coma stage of encephalopathy (defined as the loss of righting and corneal reflexes) at which time all animals had significant brain edema. Hypothermic animals (ALF-35) were sacrificed in parallel with time-matched comatose normothermic ALF animals and sham-operated controls. Brains were rapidly removed, dissected on ice and were immediately frozen in isopentane. All tissues were stored at −70 °C until use. All the above surgical methods were conducted in accordance with the Guidelines of Canadian Council of Animal care and were approved by Animal Research Committee at Saint-Luc Hospital (C.H.U.M.).

2.2. Brain water measurement

Brains were kept at 4 °C and cut into 2-mm slices. 1-mm punch biopsy specimens were obtained from the gray matter of the cerebral cortex. Water content of each specimen was measured gravimetrically using a density gradient of bromobenzene–kerosene precalibrated with K_2SO_4 as previously described (Marmarou et al., 1978). The cortical samples were placed onto the fluid column and the equilibration point was measured within 2 min. The specific gravity of the tissue was calculated and results expressed as percentage of water content. Eight measurements were made per animal, and values were arithmetically averaged.

2.3. RNA extraction

Total RNA was extracted using TRI Reagent (MRC Inc., OH) according to the manufacturer's protocol. Putative contaminating DNA was eliminated by adding 100 U of RNase-free DNase I per 50 μg of total RNA at 37 °C for 1 h. Purified RNA was then extracted with phenol, precipitated with ethanol and resuspended in diethylpyrocarbonate-treated water. RNA samples were kept at −70 °C until use.

2.4. Semi-quantitative reverse transcription-polymerase chain reaction

Expression of eNOS, occludin, caveolin, P-glycoprotein, von Willebrand factor, and endothelin-1 was investigated by standard one-step semi-quantitative RT-PCR. Total RNA (1 μg) was mixed with 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% bovine serum albumin, 200 μM dNTPs, primers at 1 μM each, AMV reverse transcriptase (80 U/ml), *Taq* DNA polymerase (20 U/ml) and 50 μCi/ml [α ^{32}P]dCTP (3000 Ci/mmol), for a total reaction volume of 50 μL. The reactions were initially heated at 50 °C for 20 min followed by PCR at 95 °C for 30 s, 60 °C for 45 s and 72 °C for 1 min. Amplification efficiency conditions were determined after a kinetic study to ensure all experiments were performed in the exponential phase of amplification where PCR products remain proportional to initial template concentration (data not shown). In all the experiments, β-actin was used as an internal standard to monitor loading variations. After amplification, samples were electrophoresed onto 9% polyacrylamide gels, dried, autoradiographed at −70 °C with an intensifying screen. Each band was excised and Cerenkov radiation was quantified using a β-counter.

Oligonucleotide primers were designed using the PRIMER3 program (Rozen and Skaletsky, 2000) at http://primer3.sourceforge.net/based on the following GeneBank accession numbers: V01217 (β-actin), X59949 (eNOS), XM_342759 (von Willebrand factor), M64711 (endothelin-1), BC161826 (Caveolin-1), AB016425 (Occludin), and L15079 (P-glycoprotein). The forward and reverse oligonucleotide primer sequences were as follows: 5′-CATCCCCCAAAGTTCTAC-3′ and 5′-CCAAAGCCTTCATACATC-3′ (β-Actin, 347 bp); 5′-TCAGCGGCTGGTACATGAG-3′ and 5′-ACAGGAAATAGTTGACCATCTC-3′ (eNOS, 351 bp); 5′-TGCTTCTTACGCCCATCTCT-3′ and 5′-CACTCATACTCTGGGCAGCA-3′ (von Willebrand factor, 444 bp); 5′-AGTGTGTCTACTTCTGCCAC-3′ and 5′-CAGCACTTCTTGTCTTTTTGG-3′ (Endothelin-1, 178 bp); 5′-ACCGCTTGCTGTCTACCATC-3′ and 5′-ATCTCT TCCTGCGTGCTGAT-3′ (Caveolin 1, 235 bp); 5′-GCTTTAATCATTGTTTTTGCTGTG-3′ and 5′-CTCTAGGTTATCGTTGCTGCTGTA-3′ (Occludin, 357 bp); 5′-CTTTGTGGTGGGGACACTCT-3′ and 5′-CGTCTGTGGCGAGTCTTGTA-3′ (P-glycoprotein, 332 bp). The specificity of the oligonucleotide primers was verified using the program BLASTN (National Center for Biotechnology Information, Bethesda, MD).

2.5. Statistical analysis

All data are expressed as the mean ± S.E.M. and statistical analysis was performed using unpaired Student's *t*-test (2 group comparisons) or one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis (multiple comparisons). A probability of $p < 0.05$ was chosen to establish significance between the groups. Data were analyzed by using Prism 4.0 software (Prism 4.0, San Diego, CA).

3. Results

Following hepatic devascularization, normothermic animals developed symptoms of encephalopathy progressing from lethargy to loss of righting and corneal reflexes (coma stage). Hypothermia significantly delayed the onset of encephalopathy so that at the time normothermic rats were comatose, hypothermic animals had not started to show significant neurological deterioration. Rats sacrificed at coma stages of encephalopathy had significantly higher brain water content ($p < 0.001$) while paired rats kept mildly hypothermic (35 °C) had brain water content equivalent to that of sham-operated control animals (Fig. 1).

RT-PCR analysis revealed a significant 1.5-fold ($p < 0.05$) increase in the steady-state level of eNOS mRNA occurring as soon as 6 h (precoma) following hepatic devascularization and reaching maximal levels at coma stage of encephalopathy (1.6-fold, $p < 0.001$). However induction of eNOS mRNA expression was prevented in hypothermic animals ($p < 0.01$) (Fig. 2).

In contrast to eNOS, expression of the mRNA encoding the tight junction protein occludin was decreased twofold ($p < 0.001$) in normothermic ALF rats at coma stages of encephalopathy but was insensitive to hypothermia (Fig. 3). Similarly, expression of von Willebrand factor, a multimeric adhesive glycoprotein, was decreased 1.5-fold ($p < 0.01$) in normothermic ALF rats at coma stages of encephalopathy and this decrease was also insensitive to hypothermia (Fig. 4). Expression of the structural protein caveolin-1, endothelin-1 and the multidrug resistance P-glycoprotein were not altered in ALF rats irrespective of body temperature (Figs. 5–7).

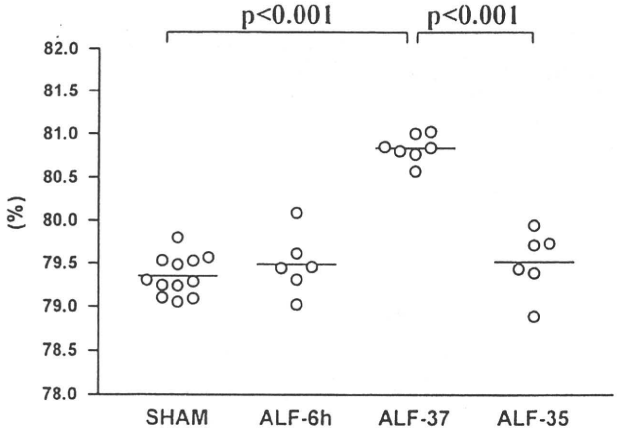

Fig. 1. Normalization of water content of cerebral cortex in ALF rats by mild hypothermia. Brain water content in rats with ALF due to hepatic devascularization compared to sham-operated controls. Normothermic ALF rats (ALF-37) had significantly higher ($p < 0.001$) brain water content compared to sham-operated controls (SHAM), ALF rats 6 h post-HAL (ALF-6 h) or hypothermic ALF rats (ALF-35). Data points represent individual animals and horizontal bars indicate mean values of $n = 6$ animals per treatment group.

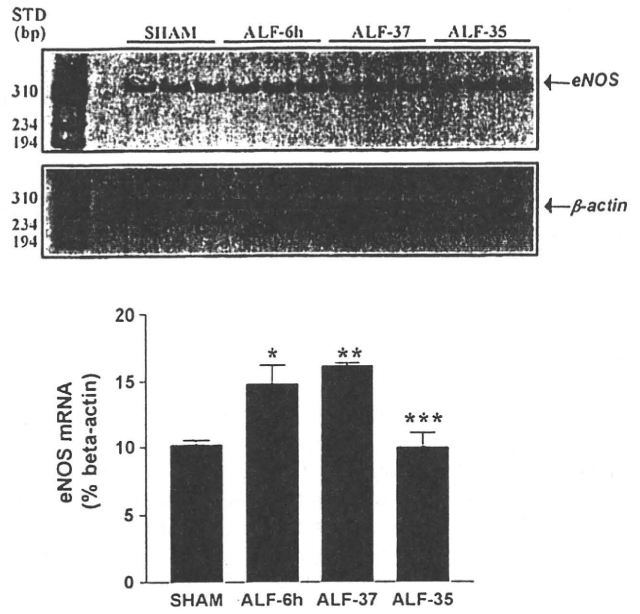

Fig. 2. Increased expression of eNOS in cerebral cortex and its prevention by mild hypothermia in experimental ALF. Endothelial nitric oxide synthase (eNOS) mRNA expression in rats with ALF due to hepatic devascularization compared to sham-operated controls. Normothermic ALF rats (ALF-6 h, ALF-37) had significantly higher steady-state levels of eNOS mRNA compared to sham-operated controls (SHAM) or hypothermic rats (ALF-35). $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. sham-operated controls; $^{***}p < 0.01$ vs. ALF-37.

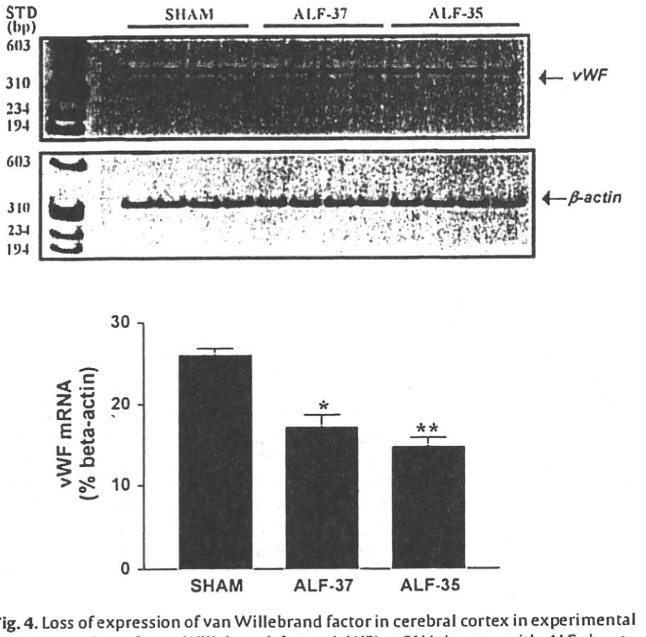

Fig. 4. Loss of expression of van Willebrand factor in cerebral cortex in experimental ALF. Expression of von Willebrand factor (vWF) mRNA in rats with ALF due to hepatic devascularization compared to sham-operated controls. Normothermic (ALF-37) and hypothermic (ALF-35) ALF rats had lower steady-state levels of vWF mRNA compared to sham-operated controls (SHAM). $^{*}p < 0.01$ and $^{**}p < 0.001$ vs. sham-operated controls.

4. Discussion

Results of the present study reveals that ALF due to hepatic devascularization leads to selective alterations in expression of genes coding for key proteins of the neurovascular unit and BBB. Coma stages of encephalopathy were accompanied by increased expression of eNOS and decreased expression of the TJ protein occludin and von Willebrand factor. No significant alterations in expression of endothelin or caveolin-1 were evident in the brains of ALF rats at comparable stages of encephalopathy. The precise mechanisms responsible for increased expression of eNOS in ALF are not completely understood. However, a previous study demonstrated that ammonia could be implicated since increased eNOS immunoreactivity was reported in the brains of portacaval shunted rats administered ammonia infusions (Blei, 2005) as well as in rats with thioacetamide-induced ALF (Hernández et al., 2004) both of which manifest brain edema and severe encephalopathy progressing to coma. Increased eNOS has the potential to cause

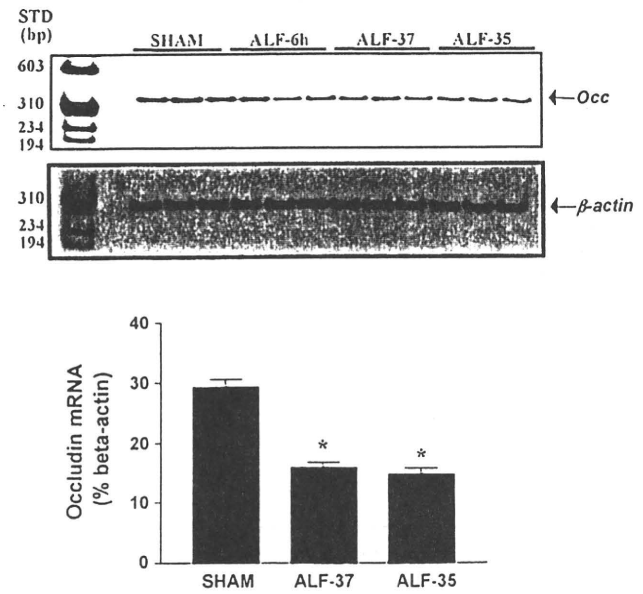

Fig. 3. Loss of expression of occludin in cerebral cortex in experimental ALF. Expression of occludin (Occ) mRNA in rats with ALF due to hepatic devascularization compared to sham-operated controls. Normothermic (ALF-37) and hypothermic (ALF-35) ALF rats had significantly lower steady-state levels of Occ mRNA compared to sham-operated controls. $^{*}p < 0.001$ vs. sham-operated controls.

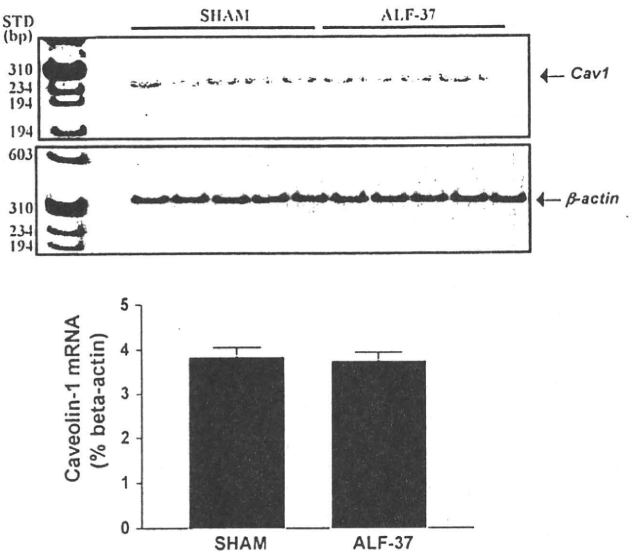

Fig. 5. Expression of caveolin in cerebral cortex in experimental ALF. Expression of Caveolin (Cav) mRNA in rats with ALF due to hepatic devascularization compared to sham-operated controls. Normothermic ALF rats (ALF-37) had similar steady-state levels of Cav mRNA compared to sham-operated controls (SHAM).

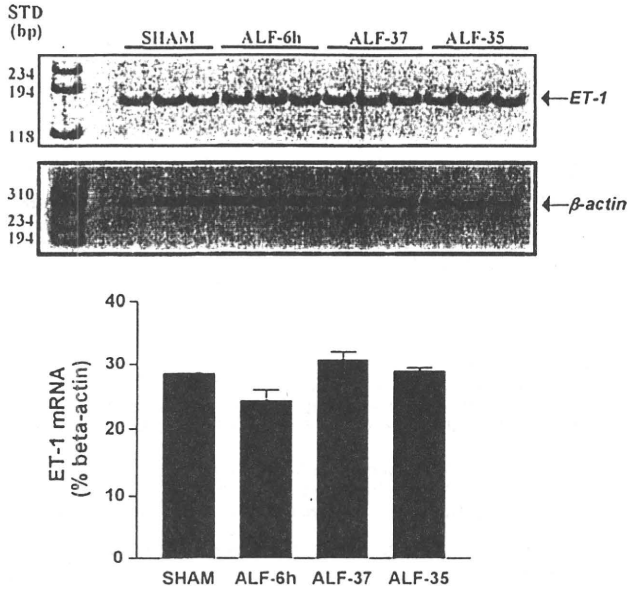

Fig. 6. Expression of endothelin-1 in cerebral cortex in experimental ALF. Expression of endothelin-1 (ET-1) mRNA in rats with ALF due to hepatic devascularization compared to sham-operated controls. Normothermic ALF rats (ALF-6 h and ALF-37) had similar steady-state levels of ET-1 mRNA compared to sham-operated controls (SHAM) or hypothermic rats (ALF-35).

increased production of nitric oxide (NO), vasodilatation and cerebral hyperemia. Increased cerebral blood flow results in increased delivery of ammonia to the brain (Ott and Larsen, 2004), a phenomenon that has been implicated in the pathogenesis of brain edema in ALF (Blei, 2001). In favor of this mechanism, administration of the vasoconstrictor indomethacin has been shown to normalize cerebral perfusion and prevent brain edema both in ALF rats (Chung et al., 2001) and in patients with ALF (Tofteng and Larsen, 2004).

Mild hypothermia completely prevented encephalopathy and brain edema in the present study and concomitantly led to normalization in expression of eNOS suggestive of a causative role. However, these conclusions are tempered by the finding of a poor

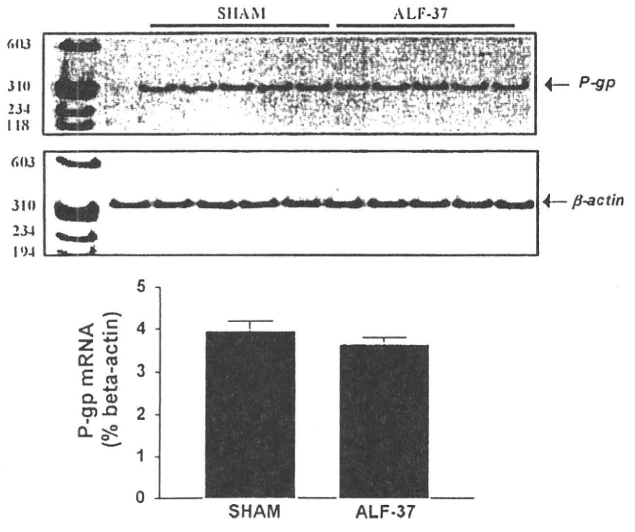

Fig. 7. Expression of P-glycoprotein in cerebral cortex in experimental ALF. Expression of P-glycoprotein P (P-gp) mRNA in rats with ALF due to hepatic devascularization compared to sham-operated controls. Normothermic ALF rats (ALF-37) had similar steady-state levels of P-gp mRNA compared to sham-operated controls (SHAM).

correlation between encephalopathy/edema at the 6 h time point suggesting that, at early stages, factors other than eNOS-derived NO also contribute to the pathogenesis of the early neurological complications of ALF. Such alternative or additional factors include brain glutamine accumulation, increased brain lactate and the presence of proinflammatory cytokines, all of which have the capacity to cause cell swelling and to contribute to the pathogenesis of HE and brain edema in ALF (Albrecht and Norenberg, 2006; Staub et al., 1990; Lazovic et al., 2005).

Loss of expression of the genes coding for the TJ protein occludin and vWF in brain extracts from ALF rats at coma stages of encephalopathy in the present study add to a growing body of evidence that the BBB is dysfunctional in ALF. Although there is no convincing evidence for physical breakdown of the barrier (Larsen et al., 1997), electron microscopic studies previously described discrete changes including swelling of both astroglia and cerebrovascular endothelial cells in the brain of ALF patients (Kato et al., 1992). Morphologic studies in hepatectomized rats reveal increases of vesicular density of the capillary endothelium (Potvin et al., 1984). Previous studies in mice exposed to the hepatotoxin azoxymethane demonstrated decreased expression of the TJ scaffolding protein zona occludens-2 and increased activity of matrix metalloproteinase-9. However, in contrast to the present study, azoxymethane-treated mice manifest clear signs of physical breakdown of the BBB including extravasation of sodium fluorescein and Evans Blue (Nguyen et al., 2006; Shimojima et al., 2008). Moreover, although results of the present study show loss of expression of occludin and vWF in the brain of hepatic devascularized rats, hypothermia sufficient to prevent edema and encephalopathy in these animals did not lead to attenuation of decreased TJ expression suggesting that these changes are not implicated in the pathogenesis of encephalopathy or brain edema in these animals. These findings underscore the notion that in this model of ALF, there is no convincing evidence that breakdown of the BBB and vasogenic edema contribute in a major way to the pathogenesis of HE and brain edema. Similar conclusions were previously reached showing electron microscopic studies in ALF patients (Kato et al., 1992).

In summary, results of the present study demonstrate selective alterations in expression of genes coding for eNOS, as well as the TJ protein occludin and vWF in the brains of rats at coma/edema stage of encephalopathy with ALF due to hepatic devascularization. Further studies will be required in order to determine the corresponding changes in proteins. Mild hypothermia sufficient to prevent encephalopathy and brain edema in these animals led to normalization of expression of eNOS but had no effect on expression of TJ proteins suggesting that cerebrovascular endothelial cell dysfunction but not BBB breakdown was implicated. At early stages of ALF, there was no significant correlation between encephalopathy/edema and increased eNOS expression suggesting the presence of additional (or alternative) mechanisms. Such mechanisms could include brain accumulation of glutamine or lactate as well as the presence of proinflammatory cytokines. These findings underscore the likely multifactorial nature of the mechanisms implicated in the pathogenesis of the neurological complications of ALF and suggest that BBB disruption is not a major feature of ALF due to liver ischemia.

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ORIGINAL PAPER

Selective alterations of brain dopamine D_2 receptor binding in cirrhotic patients: results of a ^{11}C-*N*-methylspiperone PET study

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Abstract Alterations of the brain dopamine system have been implicated in the neurological complications of chronic liver failure. The present study was aimed at the measurement of dopamine D_2 binding sites in cirrhotic patients by positron emission tomography (PET) using ^{11}C-*N*-methylspiperone as ligand. The regions of interest (ROI) were designated on a three-dimensional stereotaxic ROI template (3DSRT). The pixel values of twelve ROIs corrected by the pixel value of the cerebellum after 80 min static scanning were used to quantitate changes in binding. D_2 binding sites were significantly decreased in the hippocampus and thalamus of cirrhotic patients and were positively correlated with serum bilirubin levels and Child–Pugh scores and were negatively correlated with prothrombin times (thalamus). Loss of D_2 sites was greater in thalamus and hippocampus of alcoholic cirrhotics compared to non-alcoholics. Statistically significant correlations were also observed between D_2 binding sites in hippocampus, thalamus and lenticular nuclei and history of overt encephalopathy. These findings suggest that D_2 receptor binding in some regions of brain in cirrhotic patients is influenced by factors such as the

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severity of liver damage and history of alcohol dependency or overt encephalopathy. Alterations of D_2 receptor sites indicative of dopaminergic synaptic dysfunction could play an important role in the pathogenesis of the cognitive and motor disturbances associated with chronic liver failure.

Keywords Dopamine D_2 receptor · Positron emission tomography · Liver cirrhosis · Hepatic encephalopathy · ^{11}C-*N*-methylspiperone · Alcohol dependency

Introduction

Neuropsychiatric complications of chronic liver disease include sleep patterns and levels of consciousness as well as motor symptoms similar to those observed in Parkinson's disease. These extrapyramidal symptoms are generally believed to be attributable to disorders of dopaminergic neurotransmission in the basal ganglia (Weissenborn and Kolbe 1998; Weissenborn et al. 2000). Previous studies have revealed increased levels of dopamine metabolites (Bergeron et al. 1989) in the brains of cirrhotic patients dying in hepatic coma and reduced dopamine D_2 receptor sites in this same material (Mousseau et al. 1993). However, studies to assess the brain dopamine system in cirrhotic patients *in vivo* have so far been limited to a single case report using SPECT (Weissenborn et al. 2000).

In the present study, we examined dopamine D_2 receptor binding in cirrhotic patients using Positron Emission Tomography (PET) and the dopamine D_2 receptor ligand ^{11}C-*N*-methylspiperone (MSP). Receptor binding in these patients was assessed as a function of the severity of liver disease, patient age, prior episodes of hepatic encephalopathy (HE) and clinical laboratory parameters.

Subjects and methods

Subjects

Twenty eight patients with biopsy-confirmed cirrhosis who presented at Iwate Medical University hospital, between April 2002 and April 2005, were included in the study. Three healthy individuals served as controls, the limited number of healthy controls in the study results from the inherent high cost of PET investigations. Consequently, a stratification paradigm comparing cirrhotic patients with or without exposure to alcohol, prior episodes of HE or ascites was used in the present studies. Informed consent was obtained from all subjects (patients and controls). No patients manifested overt encephalopathy at the time of the study. Patients with overt psychiatric or neurological disorders or receiving treatment with neuroleptic drugs or with any history of exposure to psychoactive/neuroactive medication known to affect the dopaminergic system (antidepressants, antipsychotics, amphetamines, etc.) were excluded. Alcohol dependence was defined as alcohol intake of 75 g per day for five years or more.

Patient profiles are shown in Table 1. The age did not differ significantly between the healthy control subjects (mean±SD: 51.7±16.3; range 34 to 66) and the cirrhotic patients (mean±SD: 58.0±10.2; range: 41 to 75).