厚生労働科学研究費補助金
（難病・がん等の疾患分野の医療の実用化研究事業（肝炎関係研究分野））
慢性ウイルス性肝疾患の非侵襲的線化評価法の開発と臨床的有用性の確立
分担研究報告書

心拍動の変化がReal-time tissue elastography®による肝実質評価に及ぼす影響に関する検討

研究分担者　佐藤秀一　島根大学医学部附属病院　肝臓内科　講師

研究要旨

肝に歪を生ずる方法として心拍動が用いられてきたが、心拍動が変化することでRTEの解析に影響がでるかどうかをしらべた。その結果、影響を及ぼす可能性が示唆された。

A・研究目的

超音波診断装置によるReal-time tissue elastography®（以下RTE）は，組織の歪みを画像化し硬さを相対的に評価する検査法である．当初は用手圧迫により肝組織の歪みを生じさせていたが，この方法は不安定で再現性に問題があった．最近では心拍動による歪みを利用した肝線維化の非侵襲的評価法としての有用性が報告されいる．しかしながら心拍動を利用したRTEも，心機能や拍動の違いがRTEを用いた肝線維化の評価に影響を及ぼす可能性がある．今回我々は，運動負荷による心拍動の変化がRTEを用いた肝線維化の評価に影響を及ぼすか否かについて検討したので報告する．

B. 研究方法

【対象】健常ボランティア28名（男性11例，女性12例　平均年齢31.7歳，24~57歳）
BMI 21.5±2.8kg/m^2，左室駆出率 69.8±7.1%，AST 20IU/l，ALT 18IU/l，血小板 24.3 万/μl を対象とした．

【方法】超音波装置 HI VISION Preirus（日立メディコ社製）を使用．安静時および運動（ダブルマスター２階段負荷試験）直後に，右肋間よりRTEを施行した，RTEのROIは肝表面から1cm離して，血管や占拠性病変のない肝実質においた．RTE画像を6枚観察し，二人のblind readerが3枚のRTE画像を選択した．その後，日立社整の解析ソフトを用いて肝線維化と関連が高いとされている①ROI内相対的歪み値の平均値(MEAN)，②青シグナルの面積率(AREA%)，③画像の複雑度（COMP）を算出した．

C. 研究結果

心拍数（HR）は，前63.8±8.5/min，後83.8±16.1/minで運動負荷後有意に上昇していた（p<0.0001）．健常ボランティア全体ではMEANは運動負荷前116.2±11.1，運動負荷後119.3±9.7で有意差を認めなかった（p=0.23）．AREA%およびCOMPもそれぞれ運動負荷前9.1±10.0，運動負荷後6.4±6.0(p=0.15)，運動負荷前19.3±6.5，運動負荷後19.1±6.4（p=0.85）で有意差を認めなかった．しかしながら心機能が一定以上の症例に限ると，左室駆出率70%以上の群（N=14例）では，MEANは運動負荷前116±6，運動負荷後123±5（p=0.02）で有意差な上昇を認めた．

D. 考察

運動負荷による心拍出の変化は，全体としてはRTEの画像解析結果を有意に変化させなかった．しかしながら，心機能が比較的高い症例においては，運動負荷等による心拍出の変化がRTE の解析結果に影響を及ぼす可能性が示唆された．

E. 結論

運動負荷等による心拍動の変化が
Real-time Tissue Elastographyの
解析に影響を及ぼす可能性が示唆された。

F. 健康危険情報

該当なし

G. 研究発表

2.　学会発表

第84回日本超音波医学会学術集会（東京）：福間麻子，佐藤秀一，新田江里，石飛文規，花岡拓哉，飛田博史，三宅達也，柴田　宏，木下芳一．心拍動の変化がReal-time tissue elastographyによる肝実質評価に及ぼす影響．

H. 知的財産権の出願・登録状況

1. 特許取得

　　特になし

2. 実用新案登録

　　特になし

3. その他

　　特になし

厚生労働科学研究費補助金（平成23年度難病・がん等の疾患分野の医療の実用化研究事業）
分担研究報告書

慢性ウイルス性肝疾患の非侵襲的線維化評価法の開発と臨床的有用性の確立に関する研究

研究分担者　三好　久昭　香川大学医学部　消化器内科

研究要旨

非侵襲的な検査(Real-time Tissue Elastography®)で肝線維化を推定することができる。

A．研究目的

B型あるいはC型慢性肝炎および肝硬変患者に対してReal-time Tissue Elastography®にて線維化の程度を測定し肝組織診断とその結果を比較しReal-time Tissue Elastography®が肝線維化を推定出来るかどうか検討する。

B．研究方法

肝生検の前後あるいは肝切除前に血清マーカーの測定、Real-time Tissue Elastography®を行う。得られた病理組織と Real-time Tissue Elastography®の結果を対比しデータ解析を行う

(倫理面への配慮)

診療実施に関わる生データ類および同意書等を取り扱う際は被験者の秘密保護に十分配慮する。肝生検あるいは肝切除術は、さまざまなリスクを伴うが、本試験は参加の有無に関わらず肝生検あるいは肝切除術が、日常診療の一環として必要と判断される患者を対象としており、危険や不利益はないと考えられる。

C．研究結果

現在当院では 11 症例の検討を行った。今後更に症例数を増やした後詳細な検討を行っていきたい。

D．考察

超音波検査を用いるため、術者の技量、被検者の状態（高度の肥満、腹水貯留、肝癌の存在）により検査精度にばらつきが出てしまう恐れはある。しかし、これまで肝線維化を確認するには侵襲的方法しかなかったなか、非侵襲的検査であるReal-time Tissue Elastography®を用いて肝線維化を確認できることは画期的方法である。今後さらに症例を増やし、実際の病理組織と対比させてその精度を上げていく必要がある。

E．結論

肝生検や肝切除術などの侵襲的検査をせずして、非侵襲的な検査(Real-time Tissue Elastography®)で肝線維化を推定することができる。

F．研究発表

1.　論文発表　なし
2.　学会発表　なし

G．知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
なし
2. 実用新案登録
なし
3. その他
なし

厚生労働科学研究費補助金(難病・がん等の疾患分野の医療の実用化研究事業)
平成23年度慢性ウイルス性肝疾患の非侵襲的線維化評価法の開発と臨床的有用性の確立
分担研究報告書

門脈圧亢進症診断における RTE を用いた脾硬度測定の有用性

分担研究者　日浅陽一　愛媛大学大学院先端病態制御内科学講座　准教授

研究要旨:門脈圧亢進症の有無は肝硬変患者において臨床上必要不可欠な情報である。しかし門脈圧の正確な同定は侵襲的な hepatic venous pressure gradient (HVPG)によらざるをえなかった。我々は非侵襲的な検査である Real-time tissue elastography (RTE)を用いて脾硬度測定を行い、HVPG との関連を評価した。その結果、脾硬度(脾 elastic ratio)と HVPG との間には強い相関がみられ(R=0.855, P<0.0001)、肝硬度や他の肝線維化の指標よりも相関は良好であった。AUC 解析により HVPG>10mmHg として脾 elastic ratio の cut off 値を 8.24 に設定し、validation study を行ったところ、食道静脈瘤の存在診断において、94.8%と高い診断精度を示した。RTE による脾硬度(脾 elastic ratio)の測定は正確な門脈圧亢進症および食道静脈瘤の存在診断にきわめて有用と考えられる。

共同研究者
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小泉洋平　愛媛大学　助教
越智裕紀　愛媛大学　医員

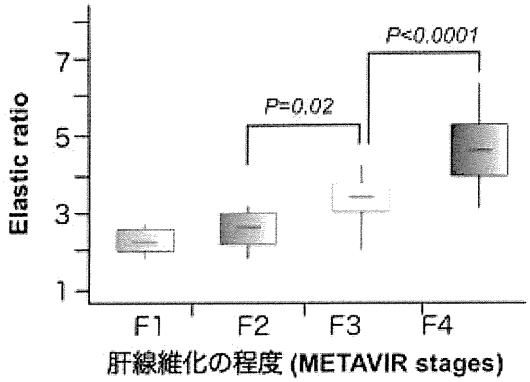
A. 研究目的

門脈圧の測定法は、門脈の直接穿刺や、門脈圧を反映する hepatic venous pressure gradient (HVPG)など検査は侵襲的である。一方、非侵襲的な方法として門脈圧亢進症診断のために、transient elastography や血液学的診断式を用いた肝硬度との関連について検討されているが、その診断能は充分ではない。門脈圧亢進症では肝硬度とともに、脾索における線維の増加などにより脾硬度も増加することが知られている。我々は超音波を用いた Real-time tissue elastography (RTE)による非侵襲的な肝線維化評価法を確立し、同検査によって得られる elastic ratio が正確で、かつ再現性が高いことを報告した (Koizumi Y, et al. Radiology 2011;258:2:610-617) (図 1)。今回の検討では、肝硬度とともに脾硬度を測定し、門脈圧との関連、門脈圧亢進症診断における有用性を明らかにすることを目的に検討した。

B. 研究方法

対象は 2009 年 1 月から 2010 年 2 月に当院に入院し、RTE による肝硬度および脾硬度測定を施行した慢性肝疾患患者 270 例。超音波診断装置は EUB-7500(日立メディコ)、探触子はリニアプローブ(EUP-L52、中心周波数 5.5MHz)を用いて、2 人の術者が測定した。まず pilot study として、60 例を対象に HVPG、肝硬度、脾硬度、ドップラーエコー、静脈瘤の有無、血液学的肝線維化診断式を評価し、HVPG と相関がみられる因子について検討を行った。肝硬度の elastic ratio は肝実質/肝静脈の elasticity の比を用い、脾硬度の elastic ratio は脾実質/脾門部静脈の elasticity の比を用いた。次いで 210 例を対象に、validation study を行い、門脈圧亢進症の診断における RTE を用いた脾硬度測定の有用性を検討した。

図1. エラストグラフィーで測定した Elastic ratioと肝線維化との関係



(倫理面への配慮)

本研究は、超音波を用いた非侵襲的検査方法を用いた。HVPG は、本研究を目的としてではなく治療の一環として血管造影を施行した事例を対象とした。研究方法、データ回収、分析については臨床倫理委員会に承認された上で、患者に関する個人情報の守秘義務、患者の権利保護等について十分に配慮し遂行した。

C. 研究結果

Pilot study 60 例、validation study 210 例の患者背景を表 1 に示す。

	Pilot study	validation study	*P* Value
年齢	68.0 ± 8.9 (35-89)	62.1 ± 12.7 (22-89)	0.092
男性	67.4 ± 7.9 (54-89)	62.2 ± 12.6 (25-89)	0.475
女性	69.1 ± 10.5 (35-80)	62.0 ± 12.8 (22-84)	0.920
男性 : 女性	39 : 21	113:97	0.138
etiology			
HBV	11	34	0.079
HCV	43	131	
Alcohol	4	16	
NAFLD	1	17	
PBC	1	12	
Noncirrhosis	12		
Child-Pugh class			
A (without cirrohosis)	46	161	0.087
B	8	28	
C	6	21	
食道静脈瘤			
Present	26	47	0.0049
Absent	34	143	

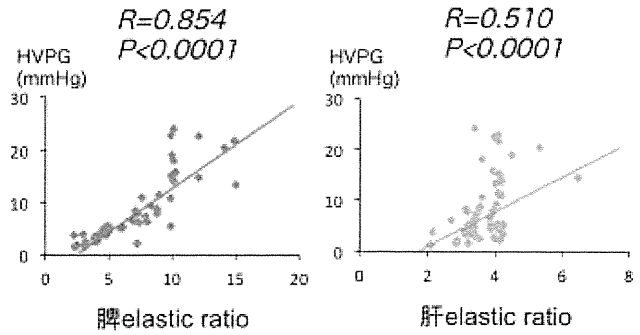
表1. 背景因子

表 2 に、HVPG と肝硬度、脾硬度の他、線維化の指標となり得る検討項目との相関関係について示す。検討項目において脾硬度は r 値が最も高く、HVPG と強い相関を示した (R=0.85, P<0.0001) (図 2)。その相関は、肝硬度よりも顕著であった。

Parameter	Mean ± SD	r Value	95% CI
HVPG (mmHg)	9.5 ± 6.1	NA	
肝硬度	3.8 ± 0.77	0.510	0.294, 0.676
脾硬度	7.6 ± 3.2	0.854	0.767, 0.911
RHA/RPV ratio	3.3 ± 1.0	0.401	0.164, 0.594
脾容積 (cm^3)	299.5 ± 183.9	0.232	-0.023, 0.459
血小板	11.1 ± 6.4	0.446	0.217, 0.629
AST/ALT ratio	1.5 ± 0.51	0.128	-0.13, 0.370
APRI	0.75 ± 0.95	0.255	0.001, 0.478
FIB-4	7.6 ± 7.3	0.335	0.089, 0.543
Congestion index	0.080 ± 0.061	0.594	0.401, 0.737
脾動脈流速(cm/s)	55.9 ± 15.0	0.047	-0.209, 0.297
脾動脈 PI	1.48 ± 0.38	0.062	-0.195, 0.311
脾動脈 RI	0.68 ± 0.20	0.216	-0.040, 0.446

表2. HVPGと各因子の相関 (pilot study)

図2. 肝・脾硬度とHVPGの相関 (pilot study)



Parameter	HVPG > 10 mmHg	HVPG > 12mmHg	Varices
肝硬度	0.832	0.781	0.833
脾硬度	0.978	0.948	0.908
RHA/RPV ratio	0.748	0.743	0.756
脾容積 (cm^3)	0.739	0.680	0.747
血小板	0.809	0.798	0.811
AST/ALT ratio	0.573	0.765	0.545
APRI	0.705	0.764	0.748
FIB-4	0.778	0.822	0.766
Congestion index	0.740	0.806	0.852
脾動脈流速(cm/s)	0.474	0.524	0.477
脾動脈 PI	0.575	0.497	0.611
脾動脈 RI	0.629	0.611	0.635

表3. 各因子の門脈圧亢進症診断能 (AUC)

各検討項目の HVPG>10 mmHg、HVPG>12 mmHg、食道静脈瘤の有無についての診断能を AUC 解析で比較した。その結果肝硬度、脾硬度、血小板数が AUC 値 0.8 以上と高い診断能を示した(表 3)。中でも、脾硬度が最も高い診断能を示した。多変量解析では、脾硬度のみが HVPG>10 mmHg (P=0.020, Odds 比 9.070)、HVPG>12 mmHg (P=0.040, Odds 比 17.708)に対して有意な寄与因子として抽出された。

AUC 解析より、脾 elastic ratio の cut off 値を HVPG>10 mmHg で 8.24、HVPG>12 mmHg で 9.99 に設定し、食道静脈瘤の有無についての診断能について、validation study を行った(表 4)。その結果、Cut off 値 8.24、9.99 のいずれの場合においても高い診断精度が得られ、脾 elastic ratio は食道静脈瘤および門脈圧亢進症の診断に有用な指標になると考えられた。

	感度(%)	特異度(%)	PPV(%)	NPV(%)	診断精度(%)
Pilot study (n=60)					
8.24	96	85	83	97	90
9.99	54	97	93	73	78
Validation study(n=210)					
8.24	98	93.8	82	99.4	94.8
9.99	26	99.4	92	82.2	82.9

表4. 脾硬度Cut off値別の門脈圧亢進症診断精度

D. 考察

RTE による脾 elastic ratio を用いた脾硬度の測定は、非侵襲的に HVPG を推定することが可能であり、食道静脈瘤と門脈圧亢進症の存在診断に有用である。脾臓は門脈圧亢進に伴い、脾索における線維の増加、血液のうっ滞、血球の増加により硬度を増すと考えられている。今回の検討により、脾硬度は肝硬度よりも強く門脈圧亢進症を反映し、測定値の再現性も良好であった。その原因として、肝硬度測定は通常右葉で測定するが、肝は部位により線維化の程度に差があることが知られている。それに対して、脾臓は解剖学的に均一な一塊として描出され、硬度に部位差が少ないことが考えられる。

非侵襲的な脾硬度測定として、Transient Elastography や MR elastography を用いた検討がある。しかし、HVPG との十分な相関は得られていない。さらに Transient Elastography は A モードのため脾臓に測定波を確実に当てるのが困難であり、MR elastography は検査費用が高額で、測定に長時間を要する。RTE による脾硬度測定は、B モードで測定部をリアルタイムに確認でき、簡便で、かつ短時間で検査を行える利点がある。Validation study において、RTE による脾 elastic ratio 8.24 以上は、94.8%と高率に食道静脈瘤を合併しており、食道静脈瘤のリスク把握に有用と考えられる。また、RTE によって簡便に HVPG を推定できることは、臨床上きわめて有用と考えられる。

E. 結論

RTE で測定した脾硬度(脾 elastic ratio)は、強く HVPG と相関する。肝硬度や他の線維化の指標よりも良好な相関を示した。RTE による脾硬度測定は、非侵襲的であり、かつ食道静脈瘤および門脈圧亢進症の診断に有用な指標となる。

F. 健康危険情報

特記すべきことなし。

G. 研究発表

1. 学会発表

廣岡昌史、平岡淳、日浅陽一他 当科における非 B 非 C 型肝癌の臨床的特徴の検討 第 47 回日本肝臓学会総会 2011.6 東京

2. 論文発表

1) Koizumi Y, Hirooka M, Hiasa Y, et al. Liver Fibrosis in patients with chronic hepatitis C: noninvasive diagnosis by means of real-time Tissue elastography -establishment of the method for measurement. Radiology. 2011:258:610-617.

2) Hirooka M, Koizumi Y, Hiasa Y, et al. Hepatic elasticity in patients with ascites: evaluation with real-time tissue elastography. AJR Am J Roentgenol. 2011:196:W766-771.

3) Hirooka M, Ochi H, Hiasa Y, et al. Splenic elasticity measured with real-time tissue elastography is a marker of portal hypertension. Radiology.

2011:261:960-968.

H. 知的財産権の出願・登録状況
「SEP (splenic elasticity for portal hypertension) score」特願 2011-130701

Ⅲ. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
里見清一，吉村健一			誰も教えてくれなかった 癌臨床試験の正しい解釈	中外医学社		2011	
吉村健一，手良向聡	イベントの発生頻度をみるだけではダメ：生存時間解析を学ぶ		Heart View 2011;15(12)			2011	60-64
吉村健一	実地医療と臨床試験 3) 臨床試験の方法論		腫瘍内科 2011;8(5)			2011	486-95

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hayaishi S, Chung H, Kudo M, Ishikawa E, Takita M, Ueada T, Kitai S, Inoue T, Yada N, Hagiwara S, Minami Y, Ueshima K	Oral branched-chaun amino acid granules reduce the incidence of hepatocellular carcinoma and improve event-free survival in patients with liver cirrhosis.	Digest Dis	29	326-332	2011
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Ⅳ. 研究成果の刊行物・別刷

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Oral Branched-Chain Amino Acid Granules Reduce the Incidence of Hepatocellular Carcinoma and Improve Event-Free Survival in Patients with Liver Cirrhosis

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Key Words
Branched-chain amino acids · Liver cirrhosis · Hepatocellular carcinoma · Event-free survival

Abstract
Background: It has been reported that branched-chain amino acid (BCAA) supplementation can improve nutritional status and prevent liver-related complications in patients with decompensated cirrhosis. We investigated the effects of oral BCAA supplementation on the incidence of hepatocellular carcinoma (HCC) and liver-related events in patients with compensated and decompensated cirrhosis. ***Methods:*** We enrolled 211 patients with cirrhosis including 152 patients with Child-Pugh A cirrhosis, but no history of HCC. Of these, 56 received oral administration of 12 g/day BCAA for ≥6 months (BCAA group), and 155 were followed-up without BCAA treatment (control group). The HCC occurrence and event-free survival rates were compared between the two groups. We used a propensity score analysis to overcome selection bias of this retrospective analysis. ***Results:*** The HCC occurrence rate was significantly lower and event-free survival rate was significantly higher in the BCAA group than in the control group. Multivariate analyses showed BCAA supplementation was significantly associated with reduced incidence of HCC (hazard ratio (HR) 0.416, 95% confidence interval (CI) 0.216–0.800, p = 0.0085). BCAA supplementation also reduced the incidence of liver-related events in patients with Child-Pugh A cirrhosis, although the difference did not reach statistical significance (HR 0.585, 95% CI 0.336–1.017, p = 0.0575). ***Conclusions:*** Oral BCAA supplementation is associated with reduced incidence of HCC in patients with cirrhosis and seems to prevent liver-related events in patients with Child-Pugh A cirrhosis.

Introduction

Protein malnutrition is a major problem among patients with cirrhosis, characterized by a decreased blood concentration of branched-chain amino acids (BCAA) (i.e., valine, leucine, and isoleucine) and an increased concentration of aromatic amino acids. BCAA deficiency is caused by enhanced uptake and consumption of BCAA by skeletal muscle for ammonia metabolism and energy generation, despite adequate daily food intake [1, 2]. BCAA supplementation has been reported to improve the nutritional status and prevent liver-related complications in patients with decompensated cirrhosis [3–5].

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Table 1. Comparison of baseline characteristics of patients

	BCAA group n = 56	Non-BCAA group n = 155	p value
Male/female ratio	23/33	92/63	0.0198
Age, years (mean ± SD)	62.5 ± 10.3	63.0 ± 11.3	0.7917
Median (range)	63.7 (43–91)	64.9 (29–84)	
Body weight, kg (mean ± SD)	59.6 ± 11.3	59.4 ± 12.2	0.9483
Median (range)	59.0 (37.1–85.0)	60.0 (34.0–101.5)	
Baseline BMI (mean ± SD)	24.1 ± 4.1	23.1 ± 3.7	0.1272
Median (range)	24.4 (16.0–34.6)	22.9 (15.1–36.1)	
Cause of liver cirrhosis HCV/HBV/nonB-nonC	34/9/15	86/14/55	0.5320
History of IFN treatment (+/–)	5/36	18/82	0.4617
Concurrent diabetes (+/–)	13/43	30/125	0.5640
Total bilirubin level, mg/dl (mean ± SD)	1.4 ± 1.0	1.3 ± 1.4	0.4150
Median (range)	1.1 (0.5–5.2)	1.0 (0.2–14.6)	
Serum albumin level, g/dl (mean ± SD)	3.4 ± 0.6	3.7 ± 0.5	<0.0001
Median (range)	3.4 (2.2–4.9)	3.7 ± 0.5	
Platelet count, $\times 10^4/mm^3$ (mean ± SD)	9.0 ± 1.9	9.1 ± 2.0	0.7054
Median (range)	9.5 (4.5–11.9)	9.2 (3.9–11.9)	
Child-Pugh grade A/B/C	29/20/7	123/29/3	<0.0001
Serum ALT level, IU/l (mean ± SD)	54.6 ± 40.4	67.6 ± 53.5	0.0636
Median (range)	43.0 (9–185)	49.0 (2–307)	
AFP (<20/≥20 ng/ml)	15/19	28/77	0.0863
PIVKA-II (<40/≥40 mAU/ml)	6/26	12/76	0.5647
Fasting blood sugar, mg/dl (mean ± SD)	132.1 ± 76.	124.9 ± 63.8	0.5321
Median (range)	105.0 (64–510)	105.0 (60–508)	

SD = Standard deviation; BMI = body mass index; IFN = interferon; ALT = alanine aminotransferase; AFP = α-fetoprotein.

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. In Japan, HCC is the third most common cause of death from cancer in males and the fifth in females. Hepatitis C virus (HCV)-related HCC accounts for 70% of all HCC cases [6]. Advanced fibrosis is one of the predisposing factors for liver carcinogenesis in patients with HCV infection [7, 8]. Among several treatment approaches intended to reduce the incidence of HCC in patients with advanced HCV infection, interferon (IFN) has been endorsed as the standard treatment of care [9–11]. However, the virological response to IFN is significantly reduced in patients with cirrhosis and infection with HCV genotype 1 [11], and recent studies showed that maintenance IFN treatment did not reduce the incidence of HCC in patients who failed prior IFN therapy [12, 13]. Recently, several in vivo studies have shown novel effects of BCAA on the suppression of carcinogenesis [14–18]. In the present study, we investigated the effects of oral BCAA supplementation on the incidence of HCC and event-free survival in patients with cirrhosis.

Patients and Methods

Patients

We retrospectively analyzed 211 patients who were diagnosed with cirrhosis but not HCC between April 1998 and July 2008 at Kinki University Hospital. The study protocol was approved by the institutional ethics review board of Kinki University. Cirrhosis was diagnosed based on the following criteria: collateral vessels, splenomegaly or ascites on imaging finding, and a platelet count of <120,000/μl. Patients with a history of HCC were excluded from this study. Patients were classified into either the BCAA group (n = 56) as those who received oral administration of 12 g/day BCAA (LIVACT Granules; Ajinomoto Co., Inc., Tokyo, Japan) for more than 6 months or a control group (n = 155) (patients who did not receive BCAA supplementation). The baseline characteristics of patients in both groups are shown in table 1.

Follow-Up and Diagnosis of HCC

Ultrasonography and serum liver function tests and measurement of tumor markers (α-fetoprotein (AFP) and des-γ-carboxyprothrombin (DCP)) were performed every 3 months. Dynamic computed tomography (CT) was performed every year.

The patients were diagnosed with HCC based on histological or reliable clinical criteria as follows: typical imaging findings and increased HCC-related tumor markers, such as serum AFP and

Table 2. Factors associated with receiving BCAA supplementation

Variables	Odds ratio	95% confidence interval		
		lower limit	upper limit	p value
Female	0.4200	0.2040	0.8640	0.0184
HCV-positive	2.2150	0.9740	5.0370	0.0578
HBV-positive	4.6010	1.4470	14.6310	0.0097
Concurrent diabetes	2.0820	0.8880	4.8830	0.0918
Serum albumin level	0.5040	0.2330	1.0890	0.0812
Child-Pugh grade	2.5060	1.1630	5.4000	0.0190

DCP levels. Typical imaging findings for HCC include a high-density mass in the arterial phase and a low-density mass in the portal phase on dynamic CT or magnetic resonance imaging (MRI). In case they did not show typical imaging findings or no increases in tumor markers, a biopsy was performed to confirm HCC diagnosis.

The incidence of HCC development was compared between the two groups. In addition, the incidence of complications of cirrhosis that needed hospitalization, such as hepatic encephalopathy, uncontrollable ascites, rupture of esophageal or gastric varices, development of HCC and infection, was compared between the two groups.

Statistical Analysis

Data are expressed as means or medians ± SD. The χ^2 test was used to assess the differences in patient distribution. Normally distributed variables were compared using Student's t test and non-normally distributed variables were compared using the Mann-Whitney U test between the two groups. Differences in the incidence of HCC were assessed by comparing the HCC occurrence rate, which was defined as the time between study entry and HCC diagnosis. Event-free survival was defined as the time between study entry and the occurrence of the liver-related events described above. The HCC occurrence rate and the event-free survival rate were estimated by the Kaplan-Meier method and differences between the two groups were analyzed by the log-rank test. Patients were censored at the end of follow-up, or when they died of causes other than liver diseases. The Cox proportional hazard model was used for multivariate analyses of factors associated with the incidence of HCC and event-free survival. $p < 0.05$ was considered statistically significant. All statistical analyses were performed with SAS software version 9.1.3 (SAS Institute, Cary, N.C., USA).

Propensity Score Analysis

Because this study was conducted retrospectively, there were significant differences between the two groups in the distribution of sex, serum albumin level and Child-Pugh grade, as shown in table 1. This result indicates the physicians more commonly prescribed BCAA granules for patients with more deteriorated liver function. Thus, we used propensity score analysis to eliminate bias toward use of BCAA. The propensity score is the probability that a patient with specific factors will receive treatment. Briefly, the results obtained by retrospective studies using a propensity score are assumed to be similar to those obtained in prospective randomized trials. A predictive model to calculate the propensity score was constructed using factors that were associated with the use of BCAA. Based on a logistic-regression analysis using all of the available variables, six factors (sex, HCV infection, hepatitis B virus (HBV) infection, diabetes, serum albumin value, and Child-Pugh grade) were identified by a stepwise selection method and were included in the model (table 2). A propensity score was assigned to each patient.

Results

Patient Characteristics Adjusted by Propensity Score

Table 3 shows the adjusted characteristics of patients in each group according to the propensity score quartile. The differences in sex, serum albumin level, and Child-Pugh grade between the BCAA and the control group disappeared after adjustment, while differences in body weight and body mass index (BMI) appeared. Body weight and BMI were both greater in the BCAA group than in the control group.

Effects of BCAA on the Incidence of HCC

Figure 1a shows the cumulative HCC occurrence curves in both groups. The cumulative HCC occurrence rates at 3, 5 and 10 years were 90, 78 and 64%, respectively, in the BCAA group, versus 69, 59 and 38% in the control group. There was a significant difference in the incidence of HCC between the two groups ($p = 0.0038$).

Effects of BCAA on the Incidence of HCC in HCV-Related Cirrhosis

Figure 1b shows the cumulative HCC occurrence curves in patients with HCV-related cirrhosis in both groups. The cumulative HCC occurrence rates at 3, 5 and 10 years were 91, 72 and 58%, respectively, in the BCAA group, and 56, 45 and 21% in the control group. There was a significant difference in the incidence of HCC between the two groups ($p = 0.001$).

Effects of BCAA on Event-Free Survival in Patients with Child-Pugh A Cirrhosis

Figure 2 shows the cumulative event-free survival curves of patients with Child-Pugh A cirrhosis in both groups. The cumulative HCC-free survival rates at 3, 5 and 10 years were 93, 60 and 28% in the BCAA group, and 71, 53 and 13% in the control group. There was a significant difference in the event-free survival rates between the two groups ($p = 0.0408$).

Table 3. Comparison of patient characteristics stratified by propensity score quartile

Variables	Group	Lowest 25%	2nd 25%	3rd 25%	Highest 25%	p value
Propensity score	BCAA	2 (3.7%)	8 (15.7%)	19 (35.8%)	27 (50.9%)	<0.0001
	non-BCAA	52 (96.3%)	43 (84.3%)	34 (64.2%)	26 (49.1%)	
Male/female	BCAA	2 (100.0%)	6 (75.0%)	5 (26.3%)	10 (37.0%)	0.6304
	non-BCAA	49 (94.2%)	23 (53.5%)	12 (35.3%)	8 (30.8%)	
Age, years	BCAA	57.0 ± 8.7	59.1 ± 8.5	65.8 ± 11.8	61.6 ± 9.6	0.8855
	non-BCAA	62.8 ± 12.2	63.8 ± 11.9	63.0 ± 10.2	62.1 ± 10.	
Body weight, kg	BCAA	74.8 ± 14.4	59.8 ± 14.4	55.0 ± 10.7	61.6 ± 9.4	0.0045
	non-BCAA	65.0 ± 8.	58.0 ± 11.0	58.4 ± 14.8	52.9 ± 12.2	
Baseline BMI	BCAA	26.3 ± 6.4	23.1 ± 4.0	22.9 ± 3.4	25.1 ± 4.4	0.0054
	non-BCAA	23.9 ± 3.2.	22.6 ± 3.0	23.4 ± 4.6	22.2 ± 4.2	
Cause of liver cirrhosis	BCAA	2 (100.0%)	3 (37.5%)	3 (15.8%)	7 (25.9%)	0.8096
	non-BCAA	27 (51.9%)	14 (32.6%)	8 (23.5%)	6 (23.1%)	
History of IFN treatment	BCAA	0 (0%)	3 (60.0%)	14 (87.5%)	19 (95.0%)	1.0000
	non-BCAA	20 (80.0%)	24 (82.8%)	19 (73.1%)	19 (95.0%)	
Concurrent diabetes	BCAA	1 (50.0%)	5 (62.5%)	16 (84.2%)	21 (77.8%)	0.1222
	non-BCAA	44 (84.6%)	39 (90.7%)	27 (79.4%)	15 (57.7%)	
Total bilirubin level, mg/dl	BCAA	0.8 ± 0.4	0.9 ± 0.3	1.2 ± 0.6	1.7 ± 1.2	0.8361
	non-BCAA	1.1 ± 0.4	1.3 ± 2.1	1.1 ± 0.5	1.7 ± 1.9	
Serum albumin level, g/dl	BCAA	4.3 ± 0.9	4.0 ± 0.5	3.6 ± 0.3	3.0 ± 0.5	0.3005
	non-BCAA	4.1 ± 0.4	3.8 ± 0.5	3.7 ± 0.4	3.1 ± 0.5	
Platelet count, $\times 10^4/mm^3$	BCAA	9.7 ± 0.1	9.0 ± 1.7	9.3 ± 1.8	8.7 ± 2.0	0.7114
	non-BCAA	9.3 ± 1.9	9.1 ± 2.1	9.2 ± 1.8	8.5 ± 2.2	
Child-Pugh grade	BCAA	2 (100.0%)	6 (75.0%)	17 (89.5%)	4 (14.8%)	0.2830
	non-BCAA	51 (98.1%)	40 (93.0%)	25 (73.5%)	7 (26.9%)	
Serum ALT level, IU/l	BCAA	24.0 ± 18.4	89.3 ± 55.2	59.2 ± 37.4	43.4 ± 32.7	0.2280
	non-BCAA	65.4 ± 45.7	74.5 ± 62.0	68.0 ± 45.1	59.9 ± 64.8	
AFP, ng/ml	BCAA	2 (100.0%)	5 (83.3%)	2 (18.2%)	10 (66.7%)	0.4320
	non-BCAA	28 (75.7%)	24 (77.4%)	16 (80.0%)	9 (52.9%)	
PIVKA-II, mAU/ml	BCAA	2 (100.0%)	6 (85.7%)	8 (88.9%)	10 (71.4%)	0.5914
	non-BCAA	26 (81.3%)	25 (96.2%)	13 (86.7%)	12 (80.0%)	
Fasting blood sugar, mg/dl	BCAA	104.5 ± 17.7	151.9 ± 52.6	107.4 ± 21.3	146.5 ± 101.9	0.2644
	non-BCAA	124.3 ± 58.2	128.4 ± 75.1	120.7 ± 50.6	126.0 ± 74.1	

BMI = Body mass index; IFN = interferon; ALT = alanine aminotransferase; AFP = α-fetoprotein.

Results of Multivariate Analyses

Table 4 shows a summary of the results of multivariate analyses using the Cox proportional hazard model. BCAA supplementation was a significant contributing factor to reduce the incidence of HCC in all patients (hazard ratio (HR) 0.416, 95% confidence interval (CI) 0.216–0.800, p = 0.0085) and in patients with HCV-related cirrhosis (HR 0.373, 95% CI 0.182–0.764, p = 0.0071). The effect of BCAA on the suppression of HCC development was slightly greater in patients with HCV-related cirrhosis than among all patients. In addition, BCAA supplementation seemed to reduce the incidence of liver-related events in patients with Child-Pugh A cirrhosis, although this did not reach statistical significance (HR 0.585, 95%

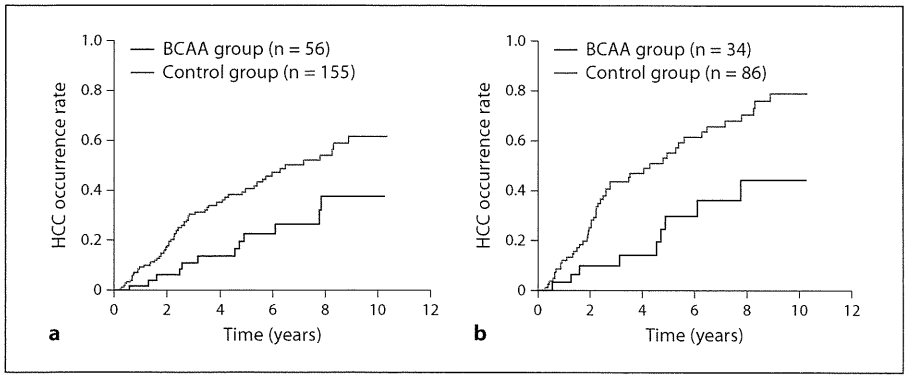

Fig. 1. a Rate of HCC occurrence. The HCC occurrence rate was significantly lower in the BCAA group than in the control group (p = 0.0038; log-rank test). **b** Rate of HCC occurrence in patients with HCV-related cirrhosis. The HCC occurrence rate was significantly lower in the BCAA group than in the control group (p = 0.001; log-rank test).

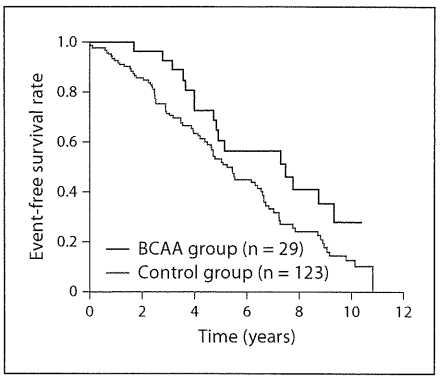

Fig. 2. Event-free survival rate in patients with Child-Pugh A cirrhosis. The event-free survival rate was significantly higher in the BCAA group than in the control group (p = 0.0408; log-rank test).

CI 0.336–1.017, p = 0.0575). The age of patients was another significant factor associated with the HCC development and the incidence of liver-related events among patients with Child-Pugh A cirrhosis.

Table 4. Summary of multivariate analyses

Variables	HR	95% CI	p value
Incidence of HCC (all cases)			
BCAA supplementation	0.416	0.216–0.800	0.0085
Propensity score	1.774	0.397–7.920	0.4526
Male sex	1.325	0.793–2.215	0.283
Age (continuous)	1.038	1.014–1.063	0.0017
Incidence of HCC (HCV)			
BCAA supplementation	0.373	0.182–0.764	0.0071
Propensity score	1.326	0.218–8.054	0.7591
Male sex	1.353	0.752–2.436	0.3133
Age (continuous)	1.032	1.000–1.064	0.0468
Event-free survival (Child-Pugh A)			
BCAA supplementation	0.585	0.336–1.017	0.0575
Propensity score	3.616	0.158–82.517	0.4206
Male sex	1.598	0.916–2.786	0.0988
Age (continuous)	1.034	1.012–1.056	0.0026

Discussion

Protein/energy malnutrition is commonly observed in patients with cirrhosis, and is represented by a decreased serum albumin level and skeletal muscle volume, and a decline in the non-protein respiratory quotient [2]. A decreased blood concentration of BCAA, caused by enhanced uptake and consumption of BCAA by skeletal muscle for ammonia metabolism and energy generation, is another manifestation of protein/energy malnutrition in patients with cirrhosis and is associated with disorders of protein synthesis and liver regeneration, and hyperammonemia [1]. Therefore, BCAA supplementation is a rational treatment for patients with cirrhosis.

Two large randomized controlled trials recently demonstrated that oral BCAA supplementation decreased the frequency of complications of cirrhosis and improved event-free survival in patients with decompensated cirrhosis [3, 4]. Based on these findings, oral BCAA supplementation is now recommended in Japanese guidelines as part of the treatment of HCV-related cirrhosis [19]. Similar to the reports mentioned above, the present study showed that the event-free survival of patients with Child-Pugh A cirrhosis was better among patients given oral BCAA supplementation than in those without, although the difference did not reach statistical significance. BCAA supplementation has also been reported to be useful as an adjuvant nutritional therapy following hepatectomy and transarterial chemoembolization, showing reduced risk of complications and better maintenance of liver function [20–22]. According to the earlier reports, BCAA seems to reduce the incidence of complications of cirrhosis by enhancing ammonia detoxification, upregulating protein synthesis and downregulating proteolysis, enhancing liver regeneration, and improving immune function [23–26].

In the present study, we did not find any beneficial effects of BCAA on the prevention of liver-related events in patients with decompensated cirrhosis, i.e. Child-Pugh B and C (data not shown). However, the relatively small number of patients classified as Child-Pugh B or C (59 of 211 patients) may account for this result. However, two other explanations seem possible. First, protein synthesis and liver regeneration, principal mechanisms involved in the improvements in liver function achieved by BCAA supplementation, are significantly aggravated in patients with decompensated cirrhosis [27]. Therefore, the benefits of therapy in terms of improvements in liver function might be limited in these patients. Second, because the liver-related events were more frequent in patients with decompensated cirrhosis than in those with compensated cirrhosis, and because BCAA supplementation does not exhibit a rapid response, the liver-related events might have occurred before the therapy induced sufficient effects in the present study. Further prospective studies are needed to address this issue.

The incidence of HCC was significantly lower in the BCAA group than in the control group (HR 0.416, 95% CI 0.216–0.800, p = 0.0085). Kobayashi et al. [28] reported that oral BCAA supplementation tended to suppress HCC development in patients with cirrhosis whose serum albumin level was <4 g/dl. Similarly, Muto et al. [29] reported that oral BCAA supplementation reduced the incidence of HCC in overweight patients (BMI $\geq$25 kg/m^2) with decompensated cirrhosis (HR 0.30, 95% CI 0.12–0.78). Based on these results, we assume that BCAA supplementation has another beneficial effect, suppressing liver carcinogenesis, in patients with cirrhosis. Although additional prospective trials are needed to confirm this novel effect of BCAA, this view is supported by several experimental and clinical studies that investigated the mechanisms underlying the anticarcinogenic effects of BCAA. According to these studies, the improvements in insulin resistance [16–18], antiangiogenesis via inhibition of vascular endothelial growth factor [15], and reduction of oxidative stress [30] that are induced by BCAA play roles in the suppression of liver carcinogenesis.

Insulin resistance, which is often observed in patients with chronic liver disease, is associated with the development of HCC [31, 32]. Kawaguchi et al. [33] demonstrated that BCAA supplementation improved insulin resistance in patients with chronic liver disease. Several in vivo studies revealed that the mechanisms underlying this effect include enhanced glucose uptake by skeletal muscle, adipocytes and hepatocytes [34–36]. These results prompted us to consider that the improvements in insulin resistance induced by BCAA supplementation play an important role in suppressing liver carcinogenesis. Although we failed to show an association between insulin resistance and liver carcinogenesis in the present study because of limited data regarding the serum insulin level, this view is supported by the finding that the effect of BCAA on the suppression of HCC development was greater among patients with HCV-related cirrhosis, which is more closely associated with insulin resistance than HBV-related cirrhosis [37].

Because this study was performed in a retrospective manner, we used propensity score analysis to reduce the effect of selection bias in the indication of BCAA supplementation. Although the differences in patient characteristics at baseline disappeared after adjustment by the propensity score, differences in body weight and BMI were found; body weight and BMI were greater in the BCAA group than in the control group. However, we believe that this does not affect the results of our study because greater body weight and BMI in patients with cirrhosis are unfavorable factors associated with an increased risk of disease progression and HCC development [32, 38].

In conclusion, we found that oral BCAA supplementation in patients with cirrhosis is associated with a reduced incidence of HCC. Oral BCAA supplementation also seems to be effective in the prevention of liver-related complications in patients with Child-Pugh A cirrhosis. Although these findings remain to be validated in prospective trials, these results advocate the necessity of BCAA supplementation in the management of patients with cirrhosis, even when liver function is compensated.

Disclosure Statement

The authors have no conflict of interest to declare.

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Correlation between hyporesponsiveness to Toll-like receptor ligands and liver dysfunction in patients with chronic hepatitis C virus infection

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SUMMARY. Hepatitis C virus (HCV)-associated antigens, such as the core and nonstructural antigens, activate host innate immune systems via Toll-like receptors (TLRs). We previously showed that chronic exposure to the core antigen induces hyporesponsiveness to TLR ligands in antigen-presenting cells via activation of TLR2 and that stimulation with TLR ligands results in impaired IL-6 production by peripheral blood monocytes from HCV-infected patients. In the present study, peripheral blood mononuclear cells (PBMCs) isolated from patients with chronic HCV or hepatitis B virus (HBV) infection were stimulated with TLR ligands to determine the production of IL-6 and IL-8 and to identify the clinical parameters associated with hyporesponsiveness to TLR ligands in patients with chronic HCV infection. The results showed that pro-inflammatory cytokine responses to TLR ligands were suppressed in PBMCs isolated from HCV-infected, but not HBV-infected, patients. The reduced cytokine responses to TLR ligands seen in HCV-infected patients correlated with platelet counts and serum prothrombin time levels. In contrast, there was no correlation between TLR-induced cytokine responses and serum levels of core antigen. Thus, hyporesponsiveness to TLR ligands in HCV-infected patients is correlated with liver dysfunction. In conclusion, both host factors and viral factors may be involved in the generation of hyporesponsiveness to TLR ligands in patients with chronic HCV infection.

Keywords: HCV core antigen, thrombocytopenia, toll-like receptor.

INTRODUCTION

Approximately 70% of patients with acute hepatitis C virus (HCV) infection do not clear the virus and go on to develop chronic infection [1]. Thus, HCV is a well-adapted human pathogen that causes persistent infection by avoiding the host immune system. HCV expresses several immunoregulatory viral proteins to evade host immune responses [2]. For example, the HCV nonstructural protein (NS) 3/4A protease blunts the innate antiviral type I IFN responses mediated via retinoic acid inducing gene-I and Toll-like receptor 3 (TLR3), both of which sense HCV RNA [3,4]. Thus, HCV NS 3/4A protease inhibits the production of type I IFNs necessary for host antiviral defence. Another HCV-associated protein with immunomodulatory properties is the HCV core antigen. Pattern recognition molecules, particularly TLRs, play a crucial role in host defence against pathogens by producing proinflammatory cytokines and antimicrobial peptides [5]. The HCV core antigen modulates TLR-mediated proinflammatory cytokine responses. Stimulation of antigen-presenting cells (APCs) with the HCV core antigen regulates innate immune responses through the activation of TLR2 [6–8]. We previously reported that chronic exposure to the HCV core antigen results in hyporesponsiveness to TLR ligands by human APCs via activation of TLR2 [8]. In addition, APCs isolated from HCV-infected patients show defective IL-6 production in response to TLR ligands, presumably because of chronic stimulation of TLR2 signalling by circulating HCV core antigen [8]. Although our previous data partially explain the molecular mechanisms by which patients with

Abbreviations: ALT, alanine aminotransferase; APC, antigen-presenting cell; BCAAs, branched-chain amino acids; ELISA, enzyme-linked immunosorbent assay; HBV, hepatitis B virus; HCV, hepatitis C virus; LPS, lipopolysaccharide; TLR, toll-like receptor; NS, nonstructural protein; PGN, peptidoglycan; PAM, Pam_3CSK4; PBMCs, peripheral blood mononuclear cells; PT, prothrombin time.

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