Table 1. 治療法による対象症例の分類

	治療群（n = 62）			無治療群（n = 18）
分類	A群（n = 24）	B群（n = 22）	C群（n = 16）	D群（n = 18）
【肝動脈化学療法】				
one shot 肝動注	+	−	+	−
low dose FP 療法	+	+	−	−

剤であり，単剤での one shot 動注療法[20]やリピオドールとの懸濁液による肝動脈化学塞栓術に使用される場合が多い[21][22]．さらに，最近ではリピオドール/アイエーコールと5-FUを併用したNew FP療法も報告されている[23]．しかし，アイエーコールの高濃度水溶液と5-FUを併用した肝動脈化学療法については報告が少ない．

今回われわれは，遠隔転移のない高度進行肝細胞癌に対する肝動脈化学療法の有効性について検討した．さらに，進行肝細胞癌の Key drug である5-FUとアイエーコールを中心とした多剤併用療法の動注レジメンを設定し，その有効性や特徴についても検討したので報告する．

I 対 象

2004年7月から2008年12月までに鹿児島厚生連病院にて腹部血管造影を施行し，治療を受けた新規肝細胞癌383例中，以下の条件にて設定された62例を治療群とした．また，同期間に同条件で自然経過を観察しえた18例（無治療例）を対照群とし，合計80例を対象とした．なお，本臨床研究は retrospective な検討であり，また，当院施設内倫理委員会による承認が得られた研究内容である．

【本研究における高度進行肝細胞癌の規定条件】

①全亜区域を主腫瘍（結節型・塊状型）または肝内転移性病変が高度に占拠する．

②両葉に広がるびまん型肝内多発症例．

③ Vp3・Vv3・B3以上の高度血管・胆管侵襲をともなう．

＊形態は Eggel 分類に準じ，①～③のいずれか1つを満たす．

＊主腫瘍の最大径や腫瘍濃染の程度は問わない．

【本研究の除外条件】

①治療歴のある症例，②遠隔転移を認める症例，③ Performance Status：Grade 3～4，④重篤な基礎疾患のある症例，⑤抗腫瘍効果を目的としたインターフェロン使用症例，⑥活動性重複癌のある症例．

規定条件③は Vp3・4により選択された症例のみであった．また，初回プロトコール不完遂例や初回治療後30日以内の死亡例は認めなかった．

II 方 法

1. 対象のグループ化

本研究は，肝動脈化学療法の治療効果を無治療例と比較すると同時に，治療内容（動注レジメン）毎の成績を比較検討した．すなわち，A群（n＝24）：one shot 肝動注＋low dose FP 併用療法，B群（n＝22）：low dose FP 単独，C群（n＝16）：one shot 肝動注単独，D群（n＝18）：無治療群に分類した（Table 1）．A，B群は low dose FP 療法を施行することより皮下埋め込み式リザーバーを留置した．A～D群の振り分けは，十分なインフォームドコンセントの後，患者および家族の意思により選択された．まず，治療希望の有無によりA～C群とD群が群別され，次に，治療希望はありながらリザーバー留置や持続動注療法に同意の得られなかった症例はC群に分類された．さらに，抗癌剤治療レジメンの選択において，濃度依存型[24]～[28]の抗癌剤を動注後，時間依存型[29]の5-FUを追加投与する one shot 肝動注＋low dose FP 多剤併用療法を試みることを選択された症例はA群へ，多剤併用療法の理論は理解されるが多施設で施行されている動注レジメンと同等の治療法を希望された症例はB群となった．

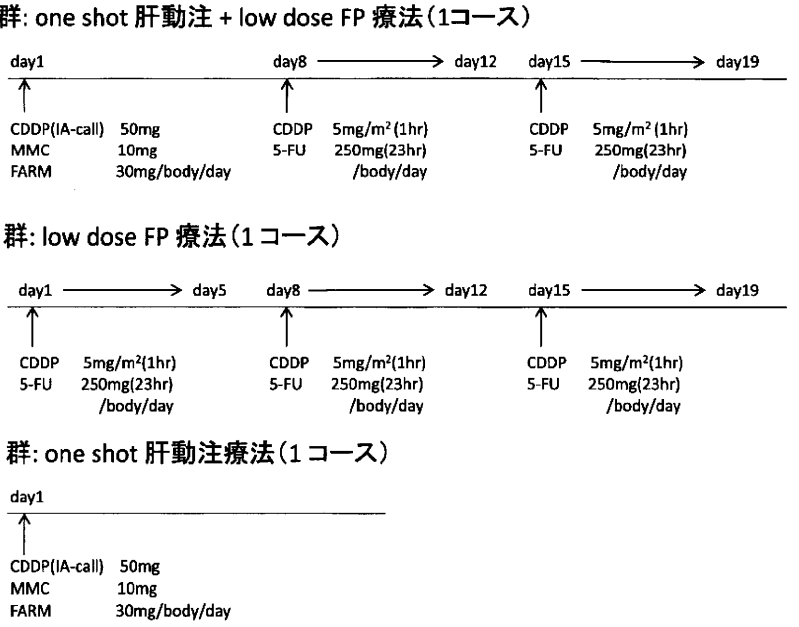

Figure 1. 高度進行肝細胞癌における肝動脈化学療法の治療レジメン.

2. 治療スケジュール

one shot 肝動注療法は Cisplatin (CDDP；IA-call) 50mg＋Mitomycin-C (MMC) 10mg＋Epirubicin (FARM) 30mg/body/day の3剤[30]を使用し，low dose FP 療法は CDDP5mg/m²＋5-fluorouracil (5-FU) 250mg/body/day を5投2休で施行した．また，A 群は one shot 肝動注の1週間後より low dose FP 療法を2週間施行し1コースとした．B 群は low dose FP 療法を3週間連続で施行し，C 群は one shot 肝動注のみを施行した (Figure 1)．肝動脈化学療法の治療効果は肝癌治療直接効果判定基準 (2004 年改定版) に準じて行った[31]．さらに，治療の中止基準は，①直接治療効果度 (1カ月後) が TE1 もしくは治療効果の総合評価 (6カ月後) が progressive disease (PD) である場合，②遠隔転移が出現し肝外病変が予後を決定すると判断された場合，③Child-Pugh 分類：12点以上 (ただしコントロール不能な肝性脳症・難治性腹水・T-Bil：4.0mg/dl 以上は12点未満でも中止)，④Performance Status：Grade 3，4かつ治療開始前に比し2以上低下した場合とし，さらに，個々の症例に応じて慎重な対応を行った．

3. 評価・検討内容

本研究では，以下の評価・検討を行った．

①患者背景因子・腫瘍背景因子の検討．

②治療群と無治療群の比較．

③肝動脈化学療法 (TAI) レジメン別の比較．

④有害事象．

統計学的処理は，患者・腫瘍背景因子の検定には，95% 信頼区間および母標本に占める比率係数を使用し検討した．累積生存率は Kaplan-Meier 法を用い Logrank 検定を行った．さらに Cox 比例ハザードモデルにて多変量解析を行い，生存に寄与する因子の検討を追加した．また，独立多群間の比較には Kruskal-Wallis 検定を用い，Bonferroni 法による補正を行った．$P<0.05$ を有意差ありとした．

III 結　果

1. 患者背景因子・腫瘍背景因子の検討

A～D 群における患者・腫瘍背景因子について，95% 信頼区間および母標本に占める比率係数を用いて検討したところ，年齢において B 群と D 群に有意差を認めた．その他，背景肝・ア

Table 2. 高度進行肝細胞癌における患者・腫瘍背景因子の検討

因子＼分類		A群（n = 24）	B群（n = 22）	C群（n = 16）	D群（n = 18）
☆年齢（歳）		70.7（66.3, 75.2）	64.2（59.8, 68.6）	65.9（60.2, 71.7）	73.6（70.0, 77.2）
●背景肝 HBV	（+/n）	0.167	0.227	0.188	0.222
HCV	（+/n）	0.458	0.364	0.563	0.500
その他	（+/n）	0.375	0.409	0.250	0.278
●飲酒歴	（+/n）	0.500	0.500	0.625	0.556
●糖尿病	（+/n）	0.292	0.364	0.188	0.278
☆T-Bil（mg/dl）		1.5（1.1, 1.8）	1.5（1.1, 1.8）	1.5（1.2, 1.9）	1.5（1.2, 1.8）
☆AST（IU/l）		87.1（61.2, 112.9）	88.9（69.7, 108.1）	96.0（69.4, 122.5）	80.7（62.4, 98.9）
☆ALT（IU/l）		67.6（42.6, 92.6）	60.3（44.6, 75.9）	72.1（46.4, 97.8）	72.2（48.8, 95.7）
☆ALB（g/dl）		3.3（3.1, 3.5）	3.3（3.1, 3.5）	3.3（3.0, 3.7）	3.2（2.9, 3.5）
☆WBC（$\times 10^3/\mu l$）		5.1（3.6, 6.7）	5.1（2.9, 7.3）	5.0（3.6, 6.3）	5.2（4.3, 6.1）
☆neutrophil（$\times 10^3/\mu l$）		3.2（2.0, 4.4）	3.4（1.8, 5.0）	3.1（2.2, 4.0）	3.2（2.6, 3.8）
☆Plt（$\times 10^4/\mu l$）		15.4（12.3, 18.4）	14.3（11.1, 17.3）	16.8（10.6, 22.9）	15.6（11.9, 19.1）
☆PT（%）		71.6（66.1, 77.1）	74.2（66.8, 81.6）	78.6（70.8, 86.3）	74.6（66.6, 82.6）
☆ICG R_{15}（%）		24.8（19.2, 30.3）	25.2（18.6, 31.9）	25.4（18.4, 32.3）	28.5（22.1, 34.9）
●腹水	（+/n）	0.333	0.409	0.500	0.389
●Child-Pugh A	（+/n）	0.458	0.455	0.438	0.389
B	（+/n）	0.375	0.363	0.375	0.500
C	（+/n）	0.167	0.182	0.187	0.111
☆AFP（$\times 10^3$ng/ml）		15.7（2.2, 29.2）	8.1（1.9, 14.4）	9.4（3.2, 15.6）	6.9（2.7, 11.2）
☆PIVKA-II（$\times 10^3$mAU/ml）		12.8（4.5, 21.1）	21.2（2.2, 40.3）	12.9（5.6, 20.2）	16.9（0.9, 32.9）
●門脈腫瘍栓（Vp3・4）	（+/n）	0.750	0.682	0.750	0.778
●最大腫瘍径					
≧50mm	（+/n）	0.542	0.364	0.625	0.500
＜50mm	（+/n）	0.458	0.636	0.375	0.500
#●腫瘍肉眼分類					
結節型，塊状型	（+/n）	0.417	0.636	0.500	0.611
びまん型	（+/n）	0.583	0.364	0.500	0.389

#：腫瘍肉眼分類はEggel分類に準ずる ☆：μ平均値（95%信頼区間下限値，上限値）で示す ●：標本数（n）に占める（+：有）の比率で示す．

ルコール・肝機能・Child-Pugh分類・腫瘍マーカー・門脈腫瘍栓・腫瘍径などに統計学的な有意差や特徴ある傾向は指摘できなかった（Table 2）．また，各群（A～D群）を男女別に分類して検討したが，性別による明確な差は認められなかった（データ記載は省略）．

2．治療群と無治療群の比較

治療群（A～C群）と無治療群（D群）の生存率（Kaplan-Meier法）の比較（Figure 2）では，無治療群に比較し治療群は有意な生存期間の延長を認めた（Logrank検定，$P<0.001$）．治療群の平均観察期間は294日（76～1288日），無治療群の平均観察期間は96日（62～158日）であった．また，無治療例では約5カ月以内で全員が死亡したが，治療例では900日を超える長期生存者が5例（8.1%）見られた．

生存に寄与する因子の解析目的で，まず，（Table 3）に示す20項目についてLogrank検定にて単変量解析を行ったところ，PT，ICG R_{15}（%），Child-Pugh分類，門脈腫瘍栓，肝動脈化学療法の5因子に有意差を認めた．この5因子に対してCox比例ハザードモデルにて多変量解析を施行したところ，ICG R_{15}（%）と肝動脈化学療法の2因子に有意差が認められた．特に肝動脈化学療法

Table 3. 高度進行肝癌における予後規定因子の検討

因子＼解析		単変量解析		多変量解析		
		Logrank 検定		Cox 比例ハザードモデル		
		n = 80	P 値	ハザード比	95%CI	P 値
年齢（歳）	≧ 70/＜ 70	42/38	0.906			
性別	男性/女性	60/20	0.482			
背景肝	HBV or HCV/その他	53/27	0.656			
飲酒歴	+/−	43/37	0.541			
糖尿病	+/−	23/57	0.756			
T-Bil（mg/dl）	≧ 1.5/＜1.5	36/44	0.055			
AST（IU/l）	≧ 80/＜80	42/38	0.722			
ALT（IU/l）	≧ 60/＜60	37/43	0.518			
ALB（g/dl）	≧ 3.5/＜ 3.5	28/52	0.353			
Plt（×10^4/μl）	≧ 15/＜ 15	40/40	0.272			
PT（%）	≧ 80/＜ 80	38/42	0.019	1.454	(0.861 ～ 2.455)	0.161
ICG R_{15}（%）	≧ 20/＜ 20	46/34	0.006	2.012	(1.095 ～ 3.698)	0.024
腹水	+/−	32/48	0.354			
Child-Pugh 分類	A/B or C	35/45	0.039	0.656	(0.346 ～ 1.241)	0.195
AFP（×10^3ng/ml）	≧ 2000/＜ 2000	36/44	0.716			
PIVKA-II（×10^3mAU/ml）	≧ 5000/＜ 5000	35/45	0.542			
門脈腫瘍栓（Vp3・4）	+/−	59/21	0.021	1.345	(0.788 ～ 2.297)	0.276
最大腫瘍径	≧50/＜50	40/40	0.708			
#腫瘍肉眼分類	結節型 or 塊状型/びまん型	43/37	0.661			
肝動脈化学療法	+/−	62/18	＜ 0.001	6.916	(3.523 ～ 13.574)	＜ 0.001

#：腫瘍肉眼分類は Eggel 分類に準ずる.

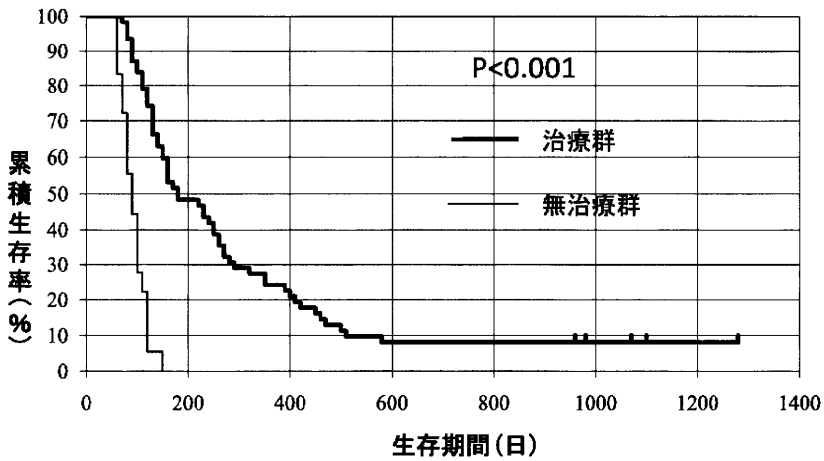

Figure 2. 高度進行肝細胞癌における治療群と無治療群の生存率の比較.

の有無はハザード比 6.916 であり，生存に寄与する最も重要な因子であることが示唆された.

3. 肝動脈化学療法（TAI）レジメンによる比較

A～D 群における生存期間は多群間 Logrank 検定で有意差を認め（P<0.001），各 2 群間の検

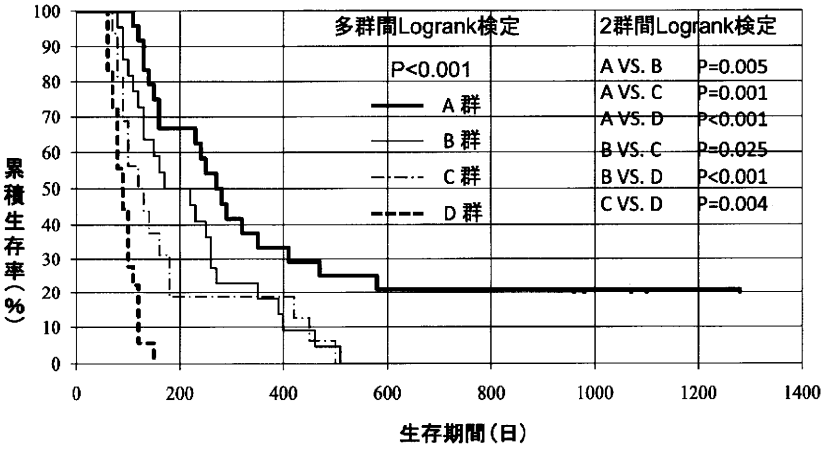

Figure 3. 肝動脈化学療法（TAI）レジメンによる高度進行肝細胞癌の生存率の比較.

Table 4. 肝動脈化学療法レジメンによる治療効果判定

		A群（n = 24）	B群（n = 22）	C群（n = 16）	P値*
1カ月後の直接治療効果度（TE）：例数	TE3	5例	0例	3例	
	TE2	19例	21例	11例	0.113
	TE1	0例	1例	2例	
6カ月後の治療効果の総合評価：例数	CR	5例$	0例	0例$	
	PR	5例	5例	3例	
	SD	5例	6例	0例	0.027
	PD	9(8)例	11(11)例	13(11)例	
奏効率％（CR + PR）		41.6％	22.7％	18.7％	0.217
腫瘍制御率％（CR + PR + SD）		62.5％†	50.0％	18.7％†	0.025

CR（著効）：complete response，PR（有効）：partial response，SD（不変）：stable disease，PD（進行）：progressive disease，*：A～C群の3群間比較(Kruskal-Wallis検定)，$：多重比較 A群とC群に有意差あり P＜0.05(Bonferroni法)，†：多重比較 A群とC群に有意差あり P＜0.05（Bonferroni法），（ ）：6カ月以内の死亡数.

定でA群が最も有意な生存期間の延長を認めた(Figure 3).

治療の効果判定は，肝癌治療直接効果判定基準(2004年改定版）に準じて行った．A～C群の治療効果について（Table 4）に示す．1カ月後の直接治療効果度は3群間に有意差を認めず（P＝0.113），TE3の効果をA群に5例（20.8％），C群に3例（18.7％）認めた（Figure 4）．B群にはTE3の症例は存在しなかった．6カ月後の治療効果の総合評価ではA～Cの3群間に有意差を認め(P＝0.027)，多重比較よりA群とC群に差を認めた．さらに，A群の奏効率は41.6％，腫瘍制御率は62.5％，B群の奏効率は22.7％，腫瘍制御率は50.0％，C群の奏効率は18.7％，腫瘍制御率は18.7％であり，奏効率・腫瘍制御率ともにA群が最も高率であった．また，B群にTE3の症例は認めなかったが，6カ月後の評価では腫瘍制御率50.0％であり，SD症例が6例（27.3％）存在した．B群は，初回直接治療効果においてはC群に及ばないが，治療効果の持続性ではC群より優れている可能性も示唆された．

TAI施行症例62例に対する予後規定因子を検討した．Table 5に示す20項目についてLogrank検定にて単変量解析を行ったところ，T-Bil，AST，Plt，ICG R_{15}（％），腹水，肝動脈化学療法の6因子に有意差を認めた．さらに，これらの

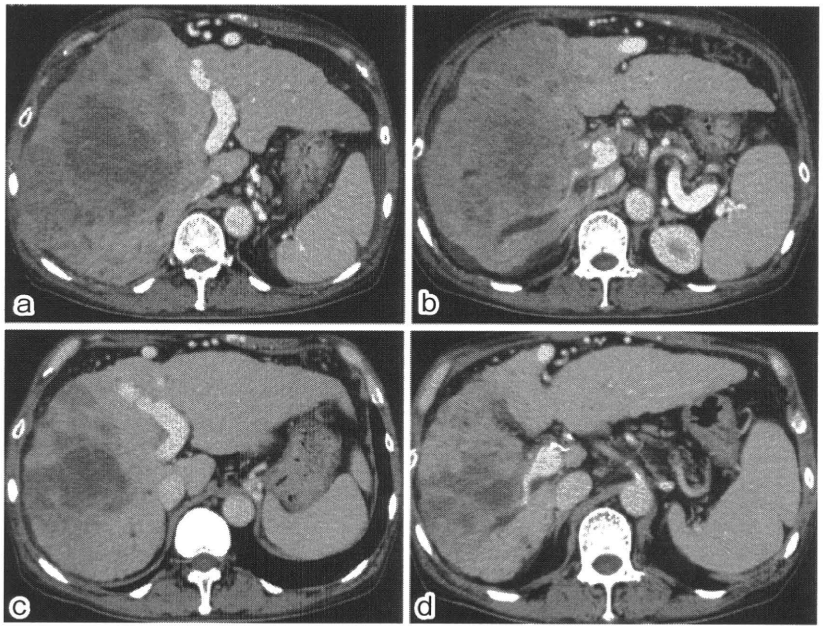

Figure 4. 腹部造影CT（初回治療終了後1カ月の直接治療効果度がTE3であった症例：A群の1例） **a**）治療前：肝右葉を中心に，内部に壊死をともなう辺縁不均一な巨大な肝細胞癌を認めた．肝臓は腫瘍により腫大していた．**b**）治療前：高度門脈腫瘍栓（Vp3・4）を認める． **c**）治療後：3剤 one shot 肝動注＋ low dose FP 療法を1コース施行し，1カ月後の評価CT．肝細胞癌は著明に縮小し，肝の突出や腫大も改善している．**d**）治療後：門脈腫瘍栓の退縮を認める．＊治療前の腫瘍マーカーは AFP：2410ng/ml/PIVKA-Ⅱ：12650mAU/ml であったが，治療後（腹部造影CTと同時日に測定した結果）は AFP：870ng/ml/PIVKA-Ⅱ：3870mAU/ml に低下していた．

6因子に対して行ったCox比例ハザードモデルによる多変量解析では肝動脈化学療法（動注レジメン）が抽出された．すなわち，one shot 肝動注＋low dose FP 多剤併用療法は生存期間の延長に寄与する独立した因子であることが示唆された．

4．有害事象

有害事象の評価は national cancer institute common terminology criteria for adverse events v3.0（CTCAE v3.0）の日本語訳 JCOG/JSCO 版[32]に従って行った．本研究で採用した治療法にはいずれも重篤な副作用の発現はなかった．非血液毒性としては発熱，消化器症状，臨床検査値の異常（肝機能・腎機能・血糖）などGrade 2以下の副作用を全例に認めた．血液毒性についても，全例で血球減少を認めたが，重篤なものや治療中断症例はなかった．ただし，one shot 肝動注療法の著効した症例では，多量の腫瘍の壊死にともない発熱の継続とCRPの高値を認めたため，抗菌薬の継続投与や low dose FP の導入が遅れた症例も存在した．また，カテーテル挿入にともなう合併症は2例存在したが（カテーテル閉塞が1例・逸脱が1例），再挿入にて治療継続は可能であった．

Ⅳ 考 察

肝細胞癌における化学療法は，肝切除やラジオ波焼灼療法，肝動脈塞栓術などの適応から外れた高度進行肝細胞癌に施行される場合が多い．しかしながら高度進行肝癌に対する肝動脈化学療法や全身化学療法については，十分な科学的根拠がないのも事実である．日本肝臓学会が2007年に提唱した〔肝細胞癌治療のアルゴリズム〕でも，高度脈管浸潤の存在する，または，びまん型に増殖した高度進行肝細胞癌に対する治療法の明確な記

Table 5. 肝動脈化学療法施行症例（A～C群）：62例における予後規定因子の検討

因子＼解析		単変量解析		多変量解析		
		Logrank 検定		Cox 比例ハザードモデル		
		n = 62	P 値	ハザード比	95%CI	P 値
年齢（歳）	≧65/＜65	30/32	0.247			
性別	男性/女性	46/16	0.328			
背景肝	HBV or HCV/その他	40/22	0.861			
飲酒歴	+/−	33/29	0.204			
糖尿病	+/−	18/44	0.197			
T-Bil（mg/dl）	≧1.5/＜1.5	28/34	0.002	1.768	(0.832～3.759)	0.138
AST（IU/l）	≧100/＜100	34/28	0.013	1.438	(0.753～2.746)	0.271
ALT（IU/l）	≧60/＜60	28/34	0.053			
ALB（g/dl）	≧3.5/＜3.5	22/40	0.152			
Plt（$\times 10^4/\mu l$）	≧15/＜15	30/32	0.047	0.984	(0.556～1.741)	0.957
PT（%）	≧80/＜80	30/32	0.236			
ICG R_{15}（%）	≧20/＜20	34/28	0.008	1.628	(0.920～2.880)	0.094
腹水	+/−	25/37	0.014	0.976	(0.538～1.771)	0.938
Child-Pugh 分類	A/B or C	28/34	0.094			
AFP（$\times 10^3$ng/ml）	≧2000/＜2000	26/36	0.513			
PIVKA-II（$\times 10^3$mAU/ml）	≧5000/＜5000	28/34	0.768			
門脈腫瘍栓（Vp3・4）	+/−	45/17	0.399			
最大腫瘍径	≧50/＜50	31/31	0.984			
#腫瘍肉眼分類	結節型 or 塊状型/びまん型	32/30	0.531			
肝動脈化学療法	A群/B＋C群	24/38	0.001	2.318	(1.281～4.196)	0.005

#：腫瘍肉眼分類はEggel分類に準ずる．

載はない．リザーバー留置による肝動脈化学療法を優先して考えると記載されているが，十分なエビデンスはない[33]．また，2005年肝癌診療ガイドライン（幕内班）でも，肝動脈化学療法や全身化学療法の有効性については明言されていない[31]．その背景には，高度進行肝細胞癌の治療において，無治療群を対照とした報告や肝動脈化学療法と全身化学療法を比較した検討は倫理的に難しい点もあるため，必然的にエビデンスレベルは低下することが考えられた．さらに，高度進行肝細胞癌の明確な定義がなく[8]，肝動脈化学塞栓術やラジオ波焼灼療法を先行した症例も含む研究では，抗癌剤耐性や肝動脈血流の変化（肝動脈の狭小化，下横隔膜動脈や肋間動脈との癒着など），肉腫様変化[35]などの問題も考えられるため，化学療法の奏効率や生存率が正しく反映されない可能性もある．そこで今回われわれは，対象とする高度進行肝細胞癌を規定し，肝動脈化学療法の有効性を無治療群と比較し検討した．さらに，進行肝細胞癌の予後因子や本研究における動注レジメンの治療効果・特徴についても追加検討した．

本研究は対象症例をA～D群に分類しretrospectiveな比較検討を行った．各群の振り分けはインフォームドコンセントの後，患者および家族の意思により決定されたものではあったが，患者自身への予後の説明に十分な統一性が図れていたとはいえず，治療法の選択に影響をきたした可能性もあると考えられた．さらに，無治療を選択された背景には，①仕事上の問題，②同じ疾患に罹患した親族の経緯により治療に積極的でない，③地理的条件，などがあげられ，年齢を含めた多数の要素が複雑に関与している可能性も推察された．

Tanaka[36]は，高度進行肝細胞癌において，どのプロトコールが有効かということより，まず，肝動脈化学療法が有用であることを科学的に検証

できる臨床研究が必要であると述べている．肝動脈化学療法施行群と無治療群をKaplan-Meier法を用いた生存率にて比較検討した結果，肝動脈化学療法は有意な生存期間の延長を認め（Figure 2），多変量解析では高度進行肝細胞癌において最も生存に寄与する因子であることが示唆された（Table 3）．本研究は症例数が非常に少なく，患者・腫瘍背景因子においても年齢に有意差を認めたことより（Table 2），症例の偏りによる影響を否定できない．しかし，すべて初回治療患者を対象とし，無治療症例と比較検討していることより，上記の結果は重要な意義をもつと考えられた．さらに，無治療では半年以内に全員が死亡するような高度進行肝癌に対して，肝動脈化学療法を施行することにより62例中5例（8.1%）はCRに至り，900日以上の長期生存を得ていることは特筆に値すると思われた．また，今回の研究では，全身化学療法との比較検討はできなかったが，全身化学療法にも著効例が多数報告されていることは十分認識すべき点であり[37][38]，今後も追加検証していくべき事項であると考えられた．

肝細胞癌は化学療法に対する感受性が低く，その標準的なレジメンは確立されていないのが現状である[39]．Furukawa[40]は肝細胞癌に対する抗癌剤の効果は，単剤より多剤併用のほうが高いと報告している．Grotheyら[41]は，進行大腸癌における予後の改善には，Key drugである5-FU，CPT-11，oxaliplatinの3剤すべてを早期より使用することが予後改善に重要であると報告している．また，杉本ら[42]も，切除不能・再発胃癌における化学療法として，S-1，CPT-11，CDDP，taxaneの4剤すべてを使い切ることが最も予後を改善すると報告している．今回われわれは，肝細胞癌のKey drugである5-FUとアイエーコールを中心とした多剤併用療法を1つの治療群として検討した．高度進行肝細胞癌は予後が非常に短くfirst-line無効時のsecond-line導入では，時間的猶予のない症例が多数存在する可能性も危惧されたため，初回治療よりone shot肝動注＋low dose FP多剤併用療法を積極的に導入する動注レジメン（A群）を，他のレジメン（B群，C群）と比較検討した（Figure 1）．生存率（Kaplan-Meier法）にてA群が最も有意な生存期間の延長を認め（Figure 3），6カ月後の治療効果の総合評価，奏効率，腫瘍制御率のいずれにおいても，A群が他の2群に比し良好な結果であった（Table 4）．また，肝動脈化学療法施行症例（A～C群）の予後規定因子の検討でもA群とB＋C群間に有意差を認めた（Table 5）．これらは高度進行肝細胞癌に対する多剤併用療法（濃度依存型＋時間依存型）の有効性を支持する結果であると考えられた．その理由として，進行肝細胞癌は組織学的にさまざまな分化度の癌細胞が混在する腫瘍であることが予測され（heterogeneityの高い細胞集団），その性質や発育過程は多岐にわたり，反応する抗癌剤もその性状により異なることが考えられる．本研究における多剤併用療法は，治療導入直後より4種類の抗癌剤を用いて，複数の作用機序（CDDPの濃度依存性抗腫瘍効果やmodulatorとしての効果など）を同時に併用するため予後の改善につながったことも示唆された[43]．また，Goldieら[44]は，「化学療法により治療効果が得られたとしても，その抗癌剤に対する耐性細胞は残り，これが急速に発育・増大すると治療が難しくなる．よって，抗癌剤耐性を獲得した細胞が臨床的に発見される前に，効果的な薬剤をより早期に併用すべきである．」と論じている（Goldie-Coldman hypothesis）．本研究の多剤併用療法は交差耐性のない（抗癌剤耐性のできる機序が異なる）CDDPと5-FUを軸とした動注レジメンを交互に繰り返すことで有効性が得られた可能性も推察された．さらに，本研究のTable 4では，初期の直接治療効果はone shot肝動注が高く，効果の持続性という面ではlow dose FP療法が優れている可能性が示唆され，2つの異なる抗腫瘍効果の治療法を併用することで即効性かつ遅効性に相乗した効果が得られたことも推考された．しかし，一方で，本研究は症例数が少なく，治療成績と画像所見（造影CTや血管造影など）の関係について検討が不十分である[45]．複数のレジメンによる併用化学療法や交替化学療法の有効性については否定的な意見もある[46]．また，今回の研究で

はインターフェロンを用いた治療法は検討されなかった．one shot 肝動注＋low dose FP 多剤併用療法は，高度進行肝細胞癌の予後を改善し得る見解を得たが，今後さらなる検討が必要であることも示唆された．

Tzoracoleftherakis[47]らは，全身化学療法と肝動注療法の比較を行い，奏功率は肝動注療法が優れていたが，生存期間には差はなかった，と報告している．また，Chung[48]や田中[36]らは，肝動注療法と無治療群の比較を行い，肝動注療法による生存期間の延長とその有効性を報告している．さらに野田[49]らは，高度進行肝癌における化学療法は，確立されたレジメンはないが，インターフェロン-α 併用 5-FU 肝動脈化学療法（FAIT）にて有効な治療効果が得られると報告している．今回のわれわれの研究では，肝動脈化学療法は無治療群に対し有意な生存期間の延長を認め，多変量解析では最も重要な予後因子であった．さらに，アイエーコール，5-FU を中心とした多剤併用療法を早期より導入することで，CR 率の高い有効な治療法になる可能性があることも示唆された．また，近年，分子標的治療薬の開発が進み，欧米諸国では高度進行肝癌治療の第一選択になりつつあるなか，多施設間で治療成績を集約・分析し，肝動脈化学療法の治療方針を確立することも急務であると考えられた．

結　語

・遠隔転移のない高度進行肝細胞癌に対する肝動脈化学療法は，無治療群に比較し有意な生存期間の延長を認め，多変量解析では最も重要な予後規定因子であった．

・高度進行肝細胞癌では，初回治療効果が予後を大きく左右し，one shot 肝動注＋low dose FP などの多剤併用肝動注療法によって予後の改善が得られる可能性も示唆された．

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Evaluation of transhepatic artery chemotherapy for highly advanced hepatocellular carcinoma

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We conducted transhepatic arterial infusion chemotherapy (TAI) was on 62 patients with highly advanced hepatocellular carcinoma without distant metastases and therapeutic outcome was compared with 18 who were untreated. TAI significantly prolonged the survival of the patients, and was the most important prognostic factor on multivariate analysis. The following 3 regimens for trans-arterial injection were compared：A, a combination of a bolus hepatic artery injection of 3 agents (cisplatin, mitomycin-C and epirubicin)＋low dose 5-fluorouracil＋cisplatin (FP)；B, low-dose FP alone；and C, bolus intrahepatic artery injection of the above 3 agents combined without FP. Regimen A yielded in the most effective survival rate, with an efficacy rate of 41.6% and a CR of about 20%. These results indicate that TAI is an effective therapeutic modality, and the dose FP combined with a bolus intrahepatic arterial infusion may improve outcomes in advanced liver cancer.

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Short Communication

Identification of a novel biomarker for oxidative stress induced by hydrogen peroxide in primary human hepatocytes using the 2-nitrobenzenesulfenyl chloride isotope labeling method

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Aim: Oxidative stress is involved in the progression of non-alcoholic steatohepatitis (NASH). However, there are few biomarkers that are easily measured and accurately reflect the disease states. The aim of this study was to identify novel oxidative stress markers using the 2-nitrobenzenesulfenyl (NBS) stable isotope labeling method and to examine the clinical utility of these diagnostic markers for NASH.

Methods: Proteins extracted from phosphate buffered saline- and hydrogen peroxide-loaded human primary hepatocyte were labeled with the [^{12}C]- and [^{13}C]-NBS reagents, respectively. Pairs of peaks with 6-Da differences in which the [^{13}C]-NBS labeling was more intense than the [^{12}C]-NBS labeling were detected by MALDI-TOF/MS and identified by MS/MS ion searching.

Results: Four pairs of peaks, m/z 1705–1711, m/z 1783–1789, m/z 1902–1908 and m/z 2790–2796, were identified as cytochrome *c* oxidase VIb (COX6B), liver carboxylesterase 1 (CES1), carbamoyl-phosphate synthase 1 (CPS1) and superoxide dismutase (MnSOD), respectively. Furthermore, serum MnSOD protein levels were significantly higher in NASH patients than in simple steatosis (SS) patients. The serum MnSOD levels tended to increase in parallel with the stage of fibrosis.

Conclusion: The NBS labeling technique was useful to identify biomarkers. Serum MnSOD may be a useful biomarker that can distinguish between SS and NASH.

Key words: 2-nitrobenzenesulfenyl, oxidative stress, MnSOD, non-alcoholic steatohepatitis

INTRODUCTION

IN SEVERAL LIVER diseases, including non-alcoholic steatohepatitis (NASH) and chronic hepatitis C (CHC), oxidative stress is a major pathogenetic event. Lipid peroxidation, free radical generation, CYP2E1 induction and mitochondrial dysfunction are known to induce oxidative stress and contribute to the progression of NASH and CHC.[1–3] Therefore, oxidative stress markers should be biomarkers that reflect the pattern and strength of oxidative stress and disease progression. Several oxidative stress markers for liver diseases including 8-hydroxy-2′-deoxyguanosine (8-OHdG), superoxide dismutase (SOD) and thioredoxin are well known. However, the clinical significance of these markers has not been fully evaluated.[4–6] Thus, oxidative stress markers that accurately reflect disease states and

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can be easily measured are necessary to accurately diagnose NASH or CHC.

In recent years, proteomic techniques, including 2-D gel electrophoresis (2-DE), have been commonly used to explore novel biomarkers. However, traditional 2-DE-based proteomic approaches are tedious and have several limitations, including reduced sensitivity and lack of quantitative results. Isotope-coded affinity tagging (ICAT) and isotope tagging for relative and absolute quantitation (iTRAQ) are the most commonly used chemical isotope labeling methods and can be used to address many of the limitations of 2-DE. In this report, we examined a novel stable isotope labeling method, the 2-nitrobenzenesulfenyl (NBS) labeling method developed by Kuyama *et al.*[7] The NBS labeling method is based on the specific binding reaction of the NBS reagent to tryptophan residues within a protein, and the 6-Da mass difference between [^{12}C]-NBS-labeled and [^{13}C]-NBS-labeled peptides generates a mass signature for all tryptophan-containing peptides.[7,8]

Here, we explored novel oxidative stress marker candidates using the NBS labeling method and identified four candidate oxidative stress markers in human primary hepatocytes including MnSOD. Furthermore, we verified the clinical significance of MnSOD as a diagnostic marker for NASH.

METHODS

Chemicals and materials

THE ^{13}CNBS® STABLE isotope labeling kit-N was purchased from Shimadzu Biotech (Kyoto, Japan). Human primary hepatocytes (a monolayer of human long-term hepatocytes), which were isolated from a 77-year-old woman, were purchased from Biopredic International (Rennes, France). 4-Hydroxycinnamic acid (CHCA) was obtained from Bruker Daltonics (Bremen, Germany) and 3-hydroxy-4-nitrobenzoic acid (3H4NBA) was purchased from Sigma Chemical (St Louis, MO, USA). Sequencing-grade modified trypsin was from Promega (Madison, WI, USA), and the protease inhibitor cocktail set III was from Calbiochem (Darmstadt, Germany).

Cell culture, NBS labeling and identification of NBS-labeled peptides

Human primary hepatocytes were cultured in a long-term culture medium.[9] Confluent human primary hepatocytes (~2×10^6 cells/12.5 cm^2 flask) were incubated for 24 h with phosphate buffered saline (PBS) or 200 μM hydrogen peroxide (H_2O_2).[10,11] Cells were washed and homogenized in 50 mM phosphate buffer, pH 8.0, containing 1% protease inhibitor cocktail set III. The NBS labeling was performed as previously described.[12,13] Briefly, both cell lysates (100 μg) treated with PBS or H_2O_2 were labeled with [^{12}C]- or [^{13}C]-NBS under acidic conditions, respectively. After labeling, the two respective conditioned protein mixtures were denatured with urea and reduced with tris(2-carboxyethyl)phosphine (TCEP) followed by alkylation with iodoacetamide. NBS-labeled proteins were digested with trypsin and eluted through phenyl sepharose using a stepwise gradient of increasing acetonitrile (10%, 15%, 20%, 25%, 30%, 35%, 40%, 45% and 50%) containing 0.1% trifluoro acetate. Next, the NBS-labeled peptides were ionized by a combined application of CHCA and 3H4NBA as described.[14,15] The mass spectral data were obtained by MALDI-TOF-TOF-MS, Autoflex II TOF/TOF (Bruker Daltonics) in positive-ion and reflectron mode. Pairs of peaks with a 6-Da difference were identified by MS/MS ion searching using tandem MS. The data set from the MS/MS ion was analyzed using the database search engine, Mascot (www.matrixscience.com), to find the closest match with known proteins/peptides in the database from the Swiss-Prot website.

Western blot analysis

Equal amounts of cell lysates from human primary hepatocytes (4 μg) were run on sodium dodecylsulfate polyacrylamide gels and electroblotted onto polyvinylidene fluoride membranes. The blots were probed with anti-cytochrome *c* oxidase VIb isoform 1 (anti-COX6B), anti-liver carboxylesterase 1 (anti-CES1), anti-carbamoyl-phosphate synthase [ammonia] mitochondrial 1 (anti-CPS1) and anti-MnSOD antibodies. After incubating the membrane with the appropriate horseradish peroxidase-conjugated secondary antibody, the reactivity was visualized using an ECL chemiluminescent detection kit (GE Healthcare Biosciences, Tokyo, Japan).

Real-time reverse transcription polymerase chain reaction (RT–PCR)

Total RNA was extracted from cells using ISOGEN (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. Samples were reverse-transcribed using the PrimeScript RT reagent Kit (TAKARA Bio, Shiga, Japan). Synthesized cDNA was amplified using SYBR Premix Ex Taq II (TAKARA Bio) and analyzed by StepOnePlus Real-Time PCR Systems and StepOne

Table 1 Identification and quantification of 2-nitrobenzenesulfenyl-labeled peak pairs

Accession no.	Protein name	Peak pairs (^{12}C–^{13}C, m/z)	Identified sequences
P14853	Cytochrome *c* oxidase subunit VIb isoform 1	1705–1711	NC**W**QNYLDFHR
P23141	Liver carboxylesterase 1 precursor	1783–1789	FTPPQPAEP**W**SFVK
P31327	Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor	1902–1908	GAEVHLVP**W**NHDFTK
P04179	Superoxide dismutase [Mn], mitochondrial precursor	2790–2796	FNGGGHINHSIF**W**TNLSPNGGGEPK

Bold and underlined characters highlight the tryptophan (W) residues in the identified peptide sequences.

Software ver. 2.0 (Applied Biosystems, Foster City, CA, USA). The cycle conditions were as follows: one cycle at 95 °C for 30 s followed by 35 cycles each at 95 °C for 5 s and 60 °C for 34 s. To normalize the amount of total RNA present in each reaction, the glyceraldehydes 3-phosphate dehydrogenase (GAPDH) gene was used as an internal standard.

Serum samples and MnSOD enzyme-linked immunosorbent assay (ELISA)

Serum samples were obtained from 20 healthy subjects, 15 simple steatosis (SS) patients and 29 NASH patients after a thorough clinical evaluation. Signed informed consent was obtained from each patient. The patients were diagnosed at University Hospital, Kyoto Prefectural University of Medicine (Kyoto, Japan) and Kagoshima University (Kagoshima, Japan). The study protocol was approved by the Ethics Committee of the Kagoshima University Hospital, the Kyoto Prefectural University of Medicine and the Miyazaki Prefectural Industrial Support Foundation. Serum MnSOD levels were measured by a Human Superoxide Dismutase 2 ELISA (AbFRONTIER, Seoul, Korea).

Statistical analysis

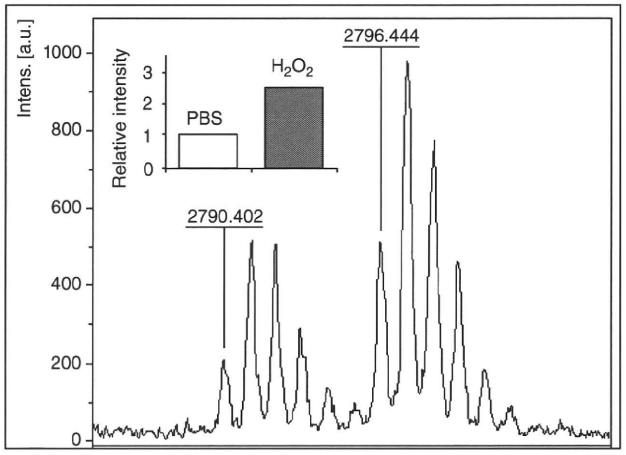
Differences among three groups were evaluated using Kruskal–Wallis test followed by Dunn's multiple comparison test. Correlation coefficients were calculated by Spearman's rank correlation analysis. A receiver-operator curve (ROC) was constructed by plotting the sensitivity and specificity (100 – specificity) for each value.

RESULTS

THE NBS-LABELED peptides from human primary hepatocytes were analyzed by MALDI-TOF/MS, and 73 pairs of peaks with 6-Da differences were detected in all mass spectra. Among these pairs of peaks, 44 pairs had a greater signal intensity in the H_2O_2-loaded sample compared to the PBS-loaded sample (data not shown). Among these 44 pairs of peaks, four peak pairs, m/z 1705–1711, m/z 1783–1789, m/z 1902–1908 and m/z 2790–2796, were identified as COX6B, CES1, CPS1 and superoxide dismutase (Mn), mitochondrial (MnSOD), respectively, by MS/MS ion searching (Table 1). The MS spectrum of the m/z 2790–2796 pair and the MS/MS spectrum of 2796 m/z ([^{13}C]-NBS labeled; MnSOD) are shown in Figure 1(a,b), respectively. Western blotting and real-time RT–PCR revealed that the protein and mRNA expression for each of these molecules increased in human primary hepatocytes after H_2O_2 loading (Fig. 1c–e).

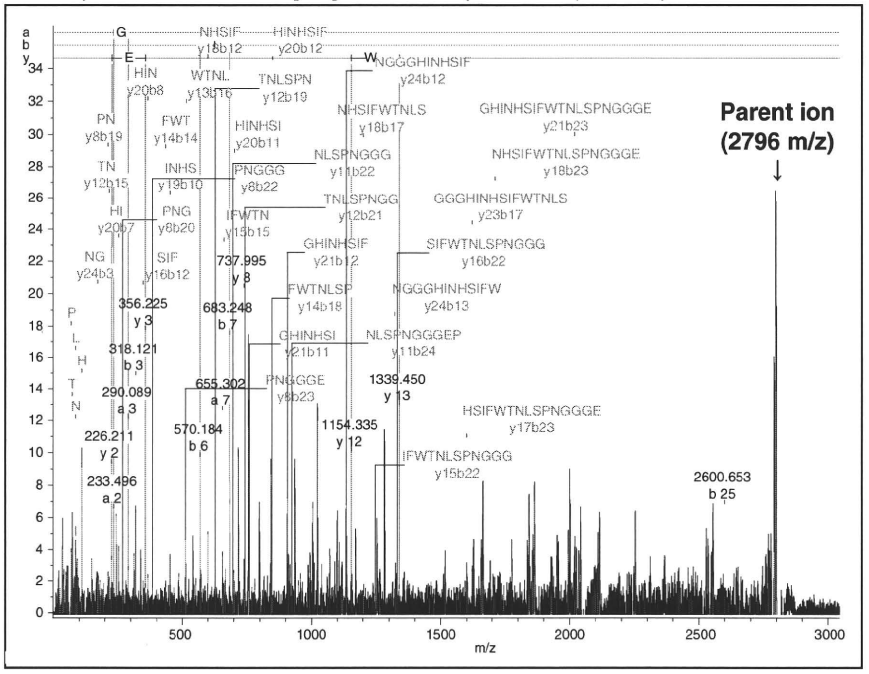
Figure 1 Typical MS spectrum and MS/MS spectra from a proteomic analysis. (a) MALDI-TOF/MS spectra of a pair of peaks, 2790–2796 m/z, and the relative intensities of the [^{13}C]-2-nitrobenzenesulfenyl (NBS)-labeled peak compared to the [^{12}C]-NBS-labeled peak. Relative intensities are the means of two independent values analyzed by Autoflex II TOF/TOF. (b) MS/MS spectra of 2796 m/z ([13C]-NBS-labeled). From the detected MS/MS spectra, superoxide dismutase (Mn) mitochondrial was identified. (c) Equal amounts of cell extracts (4 μg) from human primary hepatocytes loaded with 200 μM hydrogen peroxide (H_2O_2) for 24 h were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis and then immunoblotted with cytochrome *c* oxidase VIb isoform 1 (COX6B)-, liver carboxylesterase 1 (CES1)-, carbamoyl-phosphate synthase (ammonia), mitochondrial 1 (CPS1)-, superoxide dismutase [Mn], mitochondrial (MnSOD)- or β-actin-specific antibodies. (d) Quantitative representation of the western blot data. The results have been normalized to β-actin levels and are expressed as the levels relative to untreated cells. The data are the means of duplicate cultures. (e) The mRNA expression levels of COX6B, CES1, CPS1, MnSOD and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were measured by real-time polymerase chain reaction. The results have been normalized to GAPDH and are expressed as the levels relative to untreated cells. The data are the means of duplicate cultures. PBS, phosphate buffered saline.

(a)



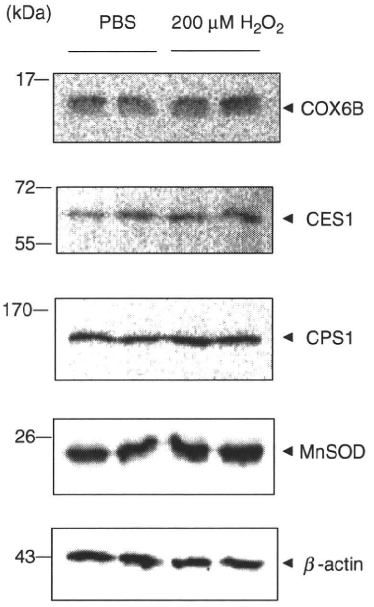
(b)

2790-2796 m/z
FNGGGHINHSIFWTNLSPNGGGEPK
Superoxide dismutase [Mn] mitcondrial precursor (P04179)



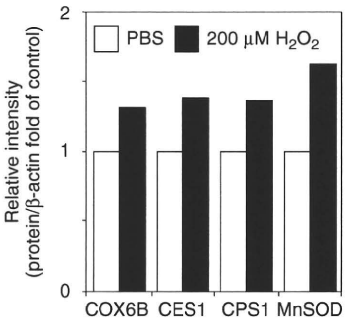
(c)

Western blotting



(d)

Densitometric analysis



(e)

Real-time RT-PCR

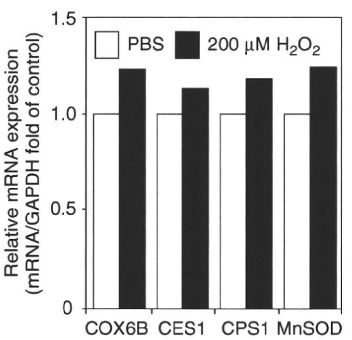


Table 2 Characteristics of study subjects

	Simple steatosis ($n = 15$)	NASH ($n = 29$)	*P*-value
Age (years)	43.2 ± 14.0	60.8 ± 14.9	**<0.001**
Sex (male/female)	11/4	11/18	**<0.05**
Height (cm)	162.5 ± 11.2	156.5 ± 8.7 (28)	0.05
Bodyweight (kg)	69.3 ± 11.8	69.2 ± 15.2 (28)	0.58
BMI (kg/m^2)	26.3 ± 3.6	28.1 ± 4.4 (28)	0.23
Diabetes (yes/no)	5/10	13/15 (28)	0.52
Hyperlipidemia (yes/no)	10/5	16/12 (28)	0.74
Hypertension (yes/no)	4/11	10/18 (28)	0.74
Hb (g/dL)	15.1 ± 1.8	14.4 ± 1.5	0.07
Plt (×10^4/μL)	23.8 ± 7.5	18.8 ± 7.0	**<0.05**
AST (IU/L)	41.6 ± 20.2	69.4 ± 46.5	**<0.05**
ALT (IU/L)	83.1 ± 53.1	94.6 ± 96.0	0.89
γ-GTP (U/L)	75.3 ± 52.4	155.6 ± 303.1	0.40
ChE (IU/L)	417.9 ± 97.5	352.8 ± 135.5	**<0.05**
γ-Glob (g/dL)	1.27 ± 0.40	1.50 ± 0.44 (24)	0.06
Total cholesterol (mg/dL)	209.2 ± 45.8	204.8 ± 52.1	0.97
Triglyceride (mg/dL)	168.3 ± 65.8	184.8 ± 168.3	0.45
BS (mg/dL)	119.1 ± 48.5	112.3 ± 34.3	0.61
Ferritin (mg/dL)	190.0 ± 112.7 (14)	239.8 ± 234.3 (22)	0.75

Values represent means ± standard deviation for the indicated number of subjects. Significant differences between the mean values ($P < 0.05$) were assessed by Fisher's exact probability test (sex, diabetes, hyperlipidemia and hypertension) or Mann–Whitney's *U*-test (other items).

Values in parentheses indicate the number of samples. Bold characters highlight statistically significant *P*-values.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BS, blood sugar; ChE, choline esterase; γ-Glob, γ-globulin; γ-GTP, γ-glutamyl transpeptidase; Hb, hemoglobin; NASH, non-alcoholic steatohepatitis; Plt, platelet count.

The clinical characteristics of the SS and NASH groups were not significantly different except for the average age, platelet count (Plt), aspartate aminotransferase (AST) and choline esterase (ChE) (Table 2). We examined the serum MnSOD levels in healthy subjects ($n = 20$), SS patients ($n = 15$) and NASH patients ($n = 29$). There were no significant differences in MnSOD serum levels between healthy subjects and SS patients (Fig. 2a). In contrast, NASH patients had significantly higher serum MnSOD levels than both healthy subjects and SS patients (Fig. 2a). In addition, as shown in Figure 2(b), the serum levels of MnSOD tended to increase in parallel with the fibrosis stage. In contrast, there was no correlation between the levels of MnSOD and the activity grade of NASH (Fig. 2c). ROC of MnSOD levels were constructed to distinguish NASH (29 patients) from SS (15 patients) (Fig. 2d). The serum MnSOD threshold level that was used to predict NASH was calculated to be 71.8 ng/mL. At this threshold, the sensitivity was 69.0% and the specificity was 80.0%. The area under the ROC (AUC) for serum MnSOD levels was 0.760 ($P = 0.010$). The ROC curves for Plt, AST and ChE, which were significantly different between SS and NASH (Table 2), were also constructed. As a result, the AUC (*P*-value, threshold, sensitivity [%], specificity [%]) for Plt, serum AST and ChE were 0.733 (0.012, 19.4, 65.5, 86.7), 0.726 (0.015, 42.0, 65.5, 73.3) and 0.687 (0.044, 317.5, 48.3, 86.7), respectively.

DISCUSSION

IN THIS REPORT, we used the NBS labeling method to identify novel oxidative stress markers in hepatocytes that can be used as diagnostic markers for NASH and identified four candidate markers, COX6B, CES1, CPS1 and MnSOD, that were upregulated with H_2O_2 loading (Table 1, Fig. 1). Several recent studies have reported novel approaches that combine the NBS labeling method with 2-DE, high-performance liquid chromatography (HPLC) and lectin column chromatographic techniques.[13–16] In our present study, we identified only four proteins, indicating that it may be necessary to modify the current method by 2-DE and column chromatographic techniques to identify additional NBS-

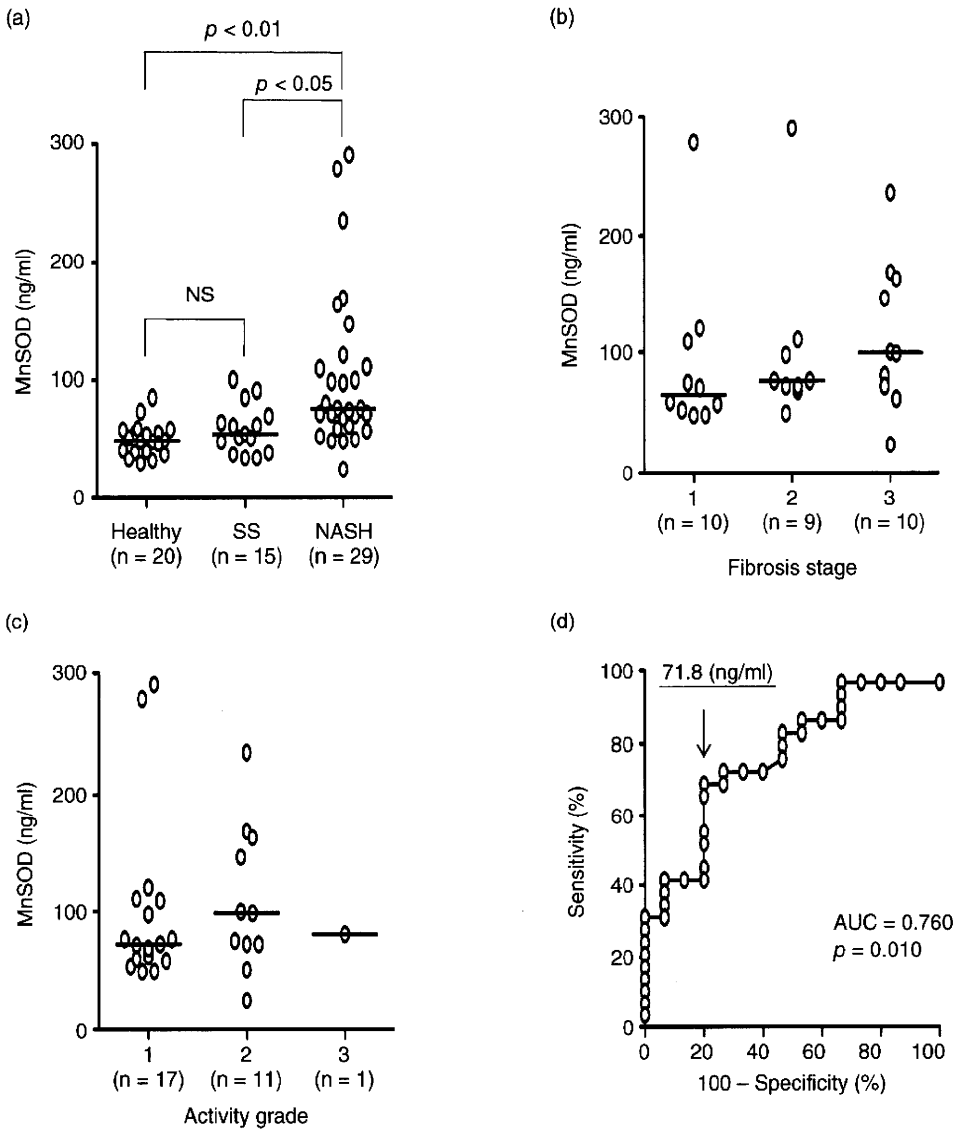


Figure 2 Clinical significance of serum MnSOD levels. (a) Serum MnSOD levels in healthy subjects and patients with SS or non-alcoholic steatohepatitis (NASH). Serum MnSOD levels were measured by enzyme-linked immunosorbent assay. (b) Comparison between serum MnSOD levels and the fibrosis stage in SS and NASH patients. (c) Comparison between serum MnSOD levels and the activity grade in SS and NASH patients. (d) Receiver–operator curve for MnSOD. The differences among three groups were evaluated using Kruskal–Wallis test followed by Dunn's multiple comparison test. Correlation coefficients were calculated by Spearman's rank correlation analysis. Bars indicate the median in the respective groups. AUC, area under the curve.

labeled peptides. In addition, further studies are needed to identify novel biomarkers by other proteomic techniques using serum samples from SS and NASH patients.

COX6B, CPS1 and MnSOD are mitochondrial proteins, and therefore may be indicators of mitochondrial disorders that are induced by oxidative stress. CPS1 is expressed primarily in the liver and small intestine and is involved in the urea cycle.[17] In galactosamine-induced rat acute hepatitis, plasma concentrations of CPS1 increase up to approximately 100-fold for 24 h after treatment.[18] This may indicate that secreted CPS1 is a serum marker for acute hepatitis. CES1, which is responsible for detoxification of exogenous compounds such

as esters, amides and thioesters, is also known to exist in the serum. Therefore, CES1, like CPS1, may be a serum oxidative stress marker.[19,20] Additional studies are needed to further evaluate the serum levels of these identified proteins.

MnSOD primarily exists in the mitochondrial matrix and eliminates reactive oxygen species (ROS) by catalyzing the dismutation of superoxide radicals and hydrogen peroxide.[19] Furthermore, MnSOD expression was previously shown to increase after exposure to hydrogen peroxide in rat hepatocytes.[21] In addition, obese mice were previously reported to have increased hepatic H_2O_2 levels and necrosis following an imbalance between increased MnSOD, which forms H_2O_2, and decreased glutathione activity, which detoxifies H_2O_2.[22] We found that MnSOD is potentially a novel diagnostic marker of NASH that can be used to distinguish between SS and NASH. One of the mechanisms contributing to increased MnSOD serum levels in NASH might be the discharge of MnSOD from necrotic hepatocytes. On the other hand, in the liver, several pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin-6 and interleukin-1β can act as common inducers of NASH.[23,24] Such pro-inflammatory cytokines have been shown to induce MnSOD expression in liver tissues.[25] Furthermore, pro-inflammatory cytokines induced the expression and secretion of MnSOD in several cancer cell lines including hepatoma cells.[26,27] In our present study, the origin of MnSOD produced in NASH and the precise mechanism of increased serum levels of MnSOD in NASH patients remain unclear. However, these reports may partially support the mechanism of MnSOD production in NASH. Further elucidation is necessary to clarify the mechanism of MnSOD expression and production in NASH.

Several reports have shown a relationship between the enzymatic activity of MnSOD and non-alcoholic fatty liver disease, NASH, liver cirrhosis and hepatocellular carcinoma.[28–31] Ono *et al.* showed that MnSOD serum levels were significantly increased in patients with primary biliary cirrhosis compared to patients with other liver diseases.[32] However, the serum protein levels of MnSOD in liver diseases have not been fully evaluated. In addition, the enzymatic activity of serum MnSOD was not different among the three groups and this activity did not correlate with serum MnSOD levels in our study (data not shown). The reasons for these results are unclear. However, as shown in Figure 2(b), serum MnSOD levels increased in parallel with the stage of fibrosis in NASH. The increase in serum MnSOD levels also significantly correlated with the serum AST levels (data not shown). These results indicate that serum MnSOD might be a biomarker that reflects hepatitic and fibrotic pathology. In addition, although MnSOD levels should increase in patients with other diseases including CHC, ROC analysis revealed that serum MnSOD may be a more sensitive biomarker than Plt, AST and ChE. We concluded that serum MnSOD is a useful biomarker that can distinguish SS and NASH.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

The complement component C3a fragment is a potential biomarker for hepatitis C virus-related hepatocellular carcinoma

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Abstract
Background Hepatocellular carcinoma (HCC) has a high mortality rate, and early detection of HCC improves patient survival. However, the molecular diagnostic markers for early HCC have not been fully elucidated. The aim of this study was to identify novel diagnostic markers for HCC.
Methods Serum protein profiles of 45 hepatitis C virus infection (HCV)-related HCC patients (HCV-HCC) were compared to 42 HCV-related chronic liver disease patients without HCC (HCV-CLD) and 21 healthy volunteers using the ProteinChip SELDI system. One of the identified proteins was evaluated as a diagnostic marker for HCC in patients with HCV.
Results Five protein peaks (4067, 4470, 7564, 7929, and 8130 m/z) had p-values less than 1×10^{-7} and were significantly increased in the sera of HCV-HCC patients compared to HCV-CLD patients and healthy volunteers. Among these proteins, an 8130 m/z peak was the most differentially expressed and identified as the complement component 3a (C3a) fragment. For HCV-HCC and HCV-CLD, the relative intensity of this C3a fragment had the best area under the ROC curve [0.70], followed by des-γ-carboxy prothrombin (DCP) [0.68], lectin-bound alpha fetoprotein (AFP-L3) [0.58] and AFP [0.53] for HCC. A combined analysis of the C3a fragment, AFP and DCP led to a 98% positive identification rate. In addition, the measurable C3a fragment in some HCC patients was not only significantly higher in the year of HCC onset compared to the pre-onset year, but also decreased after treatment.
Conclusions The 8130 m/z C3a fragment is a potential marker for the early detection of HCV-related HCC.

Keywords Hepatocellular carcinoma · Complement component C3a · Serum proteomics · Serum biomarkers · Proteinchip SELDI system · Hepatitis C virus

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Introduction

Hepatocellular carcinoma (HCC) is reportedly the third most frequent cause of global cancer-related deaths, and the incidence of HCC is increasing worldwide [1, 2]. The clearly established risk factor for HCC is chronic hepatitis C virus (HCV) infection [3].