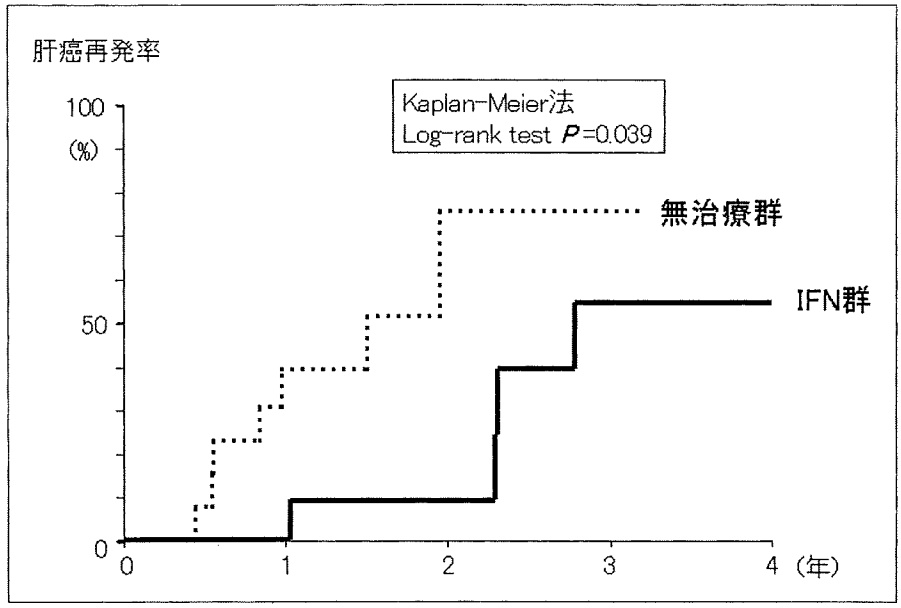

図3 C型慢性肝疾患に伴う肝細胞癌根治療法後のインターフェロン投与の影響(randomized control trial)

癌発癌に有意に関連した要因であった.

3. グリチルリチン投与の意義

インターフェロンが無効であったC型慢性肝疾患症例にグリチルリチン製剤の長期投与を行い肝癌発癌率に及ぼす影響を1,249例の多数例について検討した. インターフェロン無効例中, トランスアミナーゼが正常の2倍以上の高値症例でかつインターフェロン終了後2年以内に治療開始した症例についてみると, SNMC投与群では肝癌発癌率は有意に低くなった(P=0.021). また, 時間依存性比例ハザードモデルで発癌に寄与する要因を多変量解析で検討しても, 肝線維化などの共変量で補正してもグリチルリチン製剤使用により発癌ハザードが0.49と有意に低下し, 壊死炎症を抑制する肝庇護療法でも発癌抑制に役立つことが明らかとなった.

C型肝癌治療後のインターフェロンの再発抑制効果

C型慢性肝疾患を基礎疾患として有する肝細胞癌に外科切除などの根治的な治療を施行し, その後の再発を抑制するためにインターフェロン治療の無作為化比較試験を行った[5].

1. 対象・方法

対象は, 当院で1997年より2001年までの間に肝細胞癌に対して根治的治療が行えたC型慢性肝疾患合併肝癌34症例とした. 全例, 腹水・脳症を伴わない臨床病期Iの症例で, 年齢の中央値が61歳(51～70歳), 男女比は25：9で, 肝硬変31例・慢性肝炎3例であった. 34症例は根治的治療(外科的肝切除23例, エタノール局注療法4例, ラジオ波凝固療法7例)後に, 文書でのinformed consentを得, 中央登録方式による無作為化により, A群(インターフェロン投与群)16例とB群(経過観察群)18例とに分けられ, 肝癌再発率をprimary end-pointとして比較検討することとした. A群では1日600万単位のβ型インターフェロンを週2回静脈注射する長期投与法とした.

2. 成　績

3.9年間の観察期間に, A群から4例, B群から7例の肝癌再発がみられ, A群での肝癌再発率が低かった. 両群の累積肝癌再発率(図3)を比較すると, 1年再発率はA群0％, B群39％, 2年はA群9％, B群75％で, やはりA群で有意に再発率が低かった(log-rank test：P=0.038).

3. 評　価

ここで行ったC型肝炎ウイルスに由来する肝細胞癌に対して行った無作為化比較試験は34例と少数例の集計であるが, インターフェロン治療群では無治療群に比して明らかに低い肝癌再発率を示し, インターフェロンが肝癌再発抑制に有効であることが示された. インターフェロ

表1　C型肝炎関連肝癌治療後にインターフェロンを投与した研究報告

著者	雑誌	発表年	方法	例数	結果
Ikeda K	Hepatology	2000	RCT	20	再発率抑制(P=0.0004)
Kubo S	Ann Int Med	2001	RCT	30	再発率抑制(P=0.037)
Shiratori Y	Ann Int Med	2003	N-RCT	74	生存延長傾向
Hung CH	J Gastr Hepatol	2005	N-RCT	60	再発抑制傾向・生存延長傾向
Sakaguchi Y	Intervirology	2005	N-RCT	57	再発抑制(P=0.01)
Mazzaferro V	Hepatology	2006	RCT	150	後期再発抑制(P=0.04)

RCT：randomized control trial

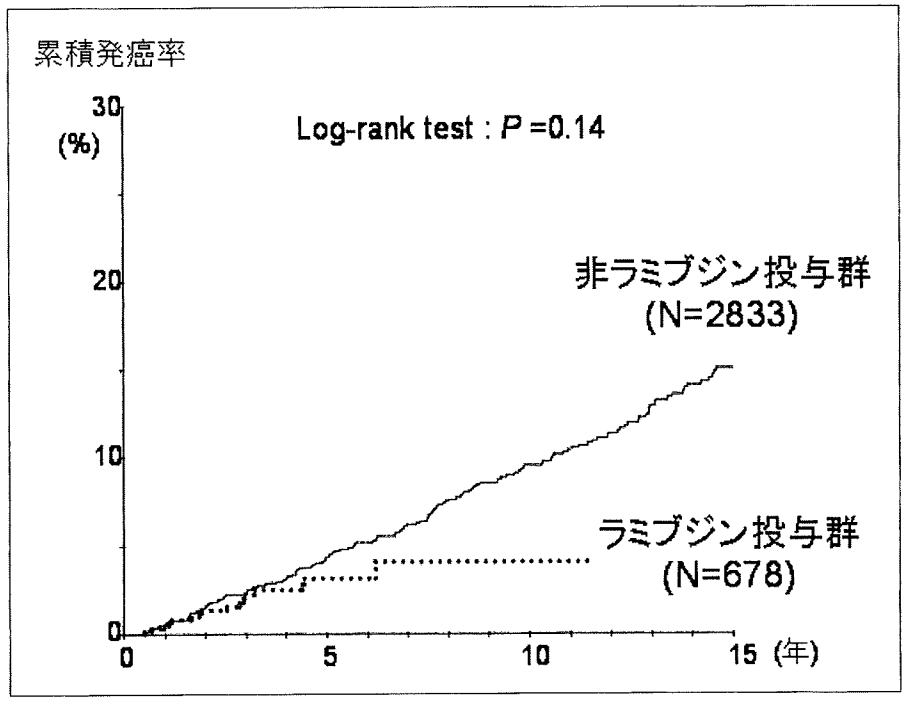

図4　ラミブジン投与別にみたB型慢性肝疾患からの粗肝癌発癌率

ン治療群での再発率曲線が有意に低いが，このインターフェロンの作用が肝細胞の壊死再生を抑制するような新規肝癌の「発癌抑制作用」だけなのか，微少な肝癌の増大を抑制するような「抗癌作用」も示しているのかなど，その作用メカニズムの早期の解明が望まれる．

これまでにわれわれの無作為化比較試験を含めて，C型肝細胞癌治療後にインターフェロン治療を行った研究が6報みられる(表1)[5)~10)]．3報は無作為化比較試験，3報はretrospective cohort studyであり，無作為化比較試験で有意に再発抑制効果がみられ，他のコホート研究では，再発抑制・生存期間延長などのエンドポイントで傾向差・有意差が報告されている．最近欧州の多施設で行われた無作為化比較試験では，全体再発率に差は示されなかったが，インターフェロンは数年経過以後の再発率を抑制すると報告している[10)]．

今後，多施設での大規模な無作為化比較試験を行い，インターフェロンの投与量や治療期間の詳細についての情報を含めて，治療効果の確認が行われることが期待される．

B型慢性肝炎に対するラミブジンの発癌抑制効果

核酸アナログ製剤のうち，長期の治療実績のあるラミブジン投与成績について，その発癌抑制効果を検討した．

対象は，当科でラミブジンを使用したB型慢性肝疾患3,674例のうち，肝細胞癌を発生していない慢性肝炎・肝硬変3,539例を対象としてretrospective cohort studyを行った．ラミブジン投与

は706例，非投与は2,833例であった．

累積粗肝細胞癌発生率を投与群・非投与群で比較すると，3年・5年・7年・10年肝癌発生率は，投与例で2.2%，3.1%，4.0%，4.0%であったが，非投与例では2.3%，4.4%，6.2%，9.5%で，投与群で粗発癌率は低い傾向であった(P=0.14)(図4)．比例ハザードモデルで肝癌発癌に寄与する独立要因は，性別(男性のハザード7.25, P=0.001)，肝線維化(F2, 3のハザード2.25, F4のハザード4.66, P<0.001)，初診年齢(35歳以上のハザード3.93, P=0.005)，初診時e抗原(陽性例のハザード2.00, P=0.005)で，これにラミブジン治療(なしのハザード1.68, P=0.084)が続いた．

当院で行ったretrospective studyでは，多変量解析でラミブジン投与例での発癌抑制傾向が示された．Liawら[11]は，651例の線維化進行B型慢性肝疾患例に対して無作為化比較試験を行い，ラミブジン投与により病変進行(非代償期移行，肝細胞癌発生，特発性細菌性腹膜炎発生，食道胃静脈瘤出血，肝疾患関連死亡)までの期間が延長するかの検討を行っている．このエンドポイントに達した人数はラミブジン群で7.8%，無治療群で17.7%であった(ハザード比 0.45, P=0.001)．エンドポイントの内訳のうち，肝癌発症率はラミブジン群3.9%，無治療群7.4%で，治療群で発癌抑制がみられた(ハザード比 0.49, P=0.047)．わが国からもretrospective cohort studyではあるものの，犬山研究会参加施設からの多数例の解析[12]で，ラミブジンによる肝癌発癌抑制のデータが示された．症例は30施設から2,795例集積され，ラミブジン投与657例，非投与2,138例が比較されている．年齢・性別・肝線維化など7項目を合致させた各群377例のcohort的研究で肝癌発癌数をみると，ラミブジン群では4例(1.1%)発癌し，年率発癌率は0.4%であったのに対し，非ラミブジン群では50例(13.3%)に発癌がみられ，その年率発癌率は2.5%であった．

B型慢性肝疾患に対するインターフェロンによる発癌率抑制効果に関しては，これまで一致した成績が出ていないが，核酸アナログ製剤による治療効果に関しても今後十分なデータが集積されることが期待されている．

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ラジオ波凝固療法と肝切除術の比較

Comparison of radiofrequency ablation with surgical resection

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◎小型少数の肝細胞癌に対して従来は外科的肝切除がもっぱら行われていたが，近年ではエタノール局注療法・マイクロ波凝固療法の時代に続き，ラジオ波凝固療法(RFA)が行われる機会が増えている．再発率はRFAでやや高いことが知られているが，外科治療と経皮的治療との間で無作為化比較試験は困難とされ，RFAが真に肝切除と同程度の治療成績が得られるかどうか，これまでのところ明らかではない．今回，3 cm以下の肝癌の初回治療として肝切除またはRFAを行った155例について長期経過観察を行った．5年後の初回再発率は，肝切除群63.6%，RFA群74.5%と高く，反復再発も頻度も高かった．しかし，最終的な生存率に寄与する要因を多変量解析で求めると，初回治療がRFAであった群での死亡ハザード比は1.05であり，生存率への影響は少なかった．

Key word：肝細胞癌，ラジオ波凝固療法(RFA)，肝切除術，再発率，生存率

肝硬変を背景にした肝癌の根治治療法としてラジオ波凝固療法(RFA)は，医学的理由に加えて，QOLおよび医療経済的にも有意義な治療として，わが国では小型結節性肝癌の標準治療のひとつとなっている．内科的なRFA治療は外科切除とは異なり，肝癌存在部位の凝固・焼灼が不十分であると腫瘍の残存から局所再発がときにみられることが知られている．このため，RFA治療を行った場合には初回再発率がわずかに高いことが指摘されている．しかし，RFA治療後の局所再発は通常，追加治療により容易にコントロールされることが多く，長期予後には影響しにくいことも報告されている．このため，RFA治療の際には，ときにみられる初回再発を重視した“初回再発率”よりも，その後の“反復再発事象”をその後の患者予後と関連づけて検討すべきであるとの考え方がみられる．

本稿では，肝切除とRFA治療を行い，根治的な治療を行った小型肝癌について長期予後を解析し，“反復再発”の実態とその後の生命予後について分析した．

ラジオ波凝固療法と肝切除術のretrospectiveな比較

1. 対象と検討方法

検討対象は根治目的で治療を行った3 cm以下3個以内の小型肝細胞癌814例のうち，1999～2003年に根治的肝切除またはRFA治療を行った155例の初発例とし，全例5年以上の観察期間が確保できる症例とした．

症例の年齢は中央値65歳(38～87歳)，男97例・女84例であった．HBs抗原陽性19例，HCV抗体陽性117例であった．肝機能ではICG 15分値の中央値26%(最小5%～最大78%)，血小板数の中央値9.9万(最小3.8万～最大25.6万)であった．

総合画像診断は，通常の腹部超音波検査，ダイナミックCT, MRIに加えてデジタルサブトラクション血管造影(DSA)，CT動脈造影(CT-HA)，CT門脈

造影(CT-AP)のすべてを行った．画像上診断できない例や非典型例では，細径針腫瘍生検で病理学的診断を行った．肝細胞癌の腫瘍径の中央値は18 mm(最小6 mm，最大30 mm)，多発例は22 mm(14.2%)であった．

これらの症例を，初回治療法が肝切除であった53例とRFAであった102例に分け，初回治療法がその後の予後に及ぼす影響を検討した．患者背景の比較にはMann-WhitneyのU検定，χ^2検定，Fisher正確確率，発癌率・生存率曲線の作成はKaplan-Meier法，発癌率・生存率の比較はlog-rank testを行った．反復して起こる事象に寄与する背景因子の多変量解析には比例ハザードモデル(Prentice-Williams-Petersonモデル)を使用した．

2. 初回治療法別の背景因子の比較

初回治療法が肝切除であった53例と，RFAであった102例の患者背景は，年齢の中央値がそれぞれ64歳，66歳($p=0.041$)で，前者で有意に若年であったが，男女比に差はなかった．HBs抗原陽性例は前者で14例(26.4%)と高く，RFAの15例(14.6%)より高かった．肝機能で比較すると，肝切除例はICG 15分値の中央値が18%であったのに対しRFA例では30%と，前者で有意に低かった($p=0.0001$)．さらに，血小板数は前者が14.4万であったが，後者は8.7万と有意に低かった($p<0.0001$)．

腫瘍径はそれぞれ20 mm，18 mmで有意差はなく，腫瘍多発に関しても6例(11.3%)，15例(14.7%)と差はなかった．

3. 肝癌治療後の初回再発率

初回治療法別に累積粗初回再発率を求めた．肝切除群・RFA群での1年再発率は9.4%，17.8%，2年は30.6%，49.7%，3年は42.1%，67.4%，4年は51.8%，74.4%，5年は60.1%，78.6%で，肝切除群での局所再発率は有意に低かった(log-rankテスト，$p=0.0020$)(図1)．初回RFA治療群で有意に肝障害が進行した症例が多かったため，両群でICG 15分値が30%未満の症例のみで初回再発率を比較した．肝切除群・RFA群での1年再発率は7.9%，15.7%，2年は28.9%，49.8%，3年は39.5%，73.3%，4年は55.3%，74.3%，5年は63.6%，74.3%で，RFA群での5年局所再発率は肝切除群より10%高いのみであった(log-rankテスト，$p=0.071$)．初回再発に寄与する独立要因を比例ハザードモデルで求めると，HBs抗原陽性(ハザード比0.52，$p=0.029$)，初回治療法(RFAは1.76，$p=0.016$)であった．

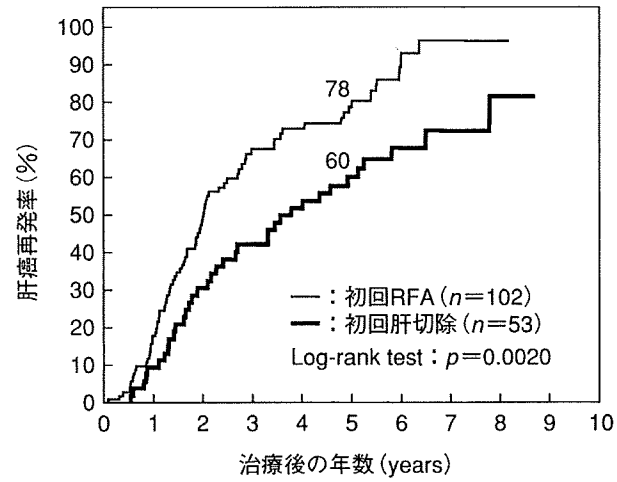

図1 “根治治療”後の小型肝癌の初回再発率

4. 肝癌治療後の再発とQOLからみた治療の実態

初回治療法別に，観察期間に何回の再発治療が行われたかを比較した．肝切除例では再治療として肝切除5回，RFAを主とする局所治療54回，肝動脈化学塞栓療法(TACE)64回，ほか6回であった．一方，RFA治療例では再治療として肝切除11回，局所治療105回，TACE 180回，ほか38回であった(図2)．

QOLの観点を含め，両群での再発実態を比較した．両群での観察期間の中央値は，肝切除群は5.7年，RFA群は4.9年で，前者でやや長かった．初発・再発を含めた観察期間内の肝癌治療回数は，1人当りそれぞれ3.42回，4.27回で，RFA治療開始群に高い傾向であった．これを1人当り年間治療回数でみると0.60回，0.87回であった．これを1999～2003年の平均在院日数で計算すると，肝切除群での総入院日数は106.3日であったのに対し，RFA群では96.3日とわずかに少なかった．

5. 反復再発事象を目的変数とする多変量解析

反復再発事象を対象とする解析のうち，j回目のイベントに対するリスク集合は(j-1)回目のイベントを起こした対象者に限るとする条件付きモデル(Prentice-Williams-Petersonモデル，Total time model)で多変量解析を行った．根治治療後の繰返

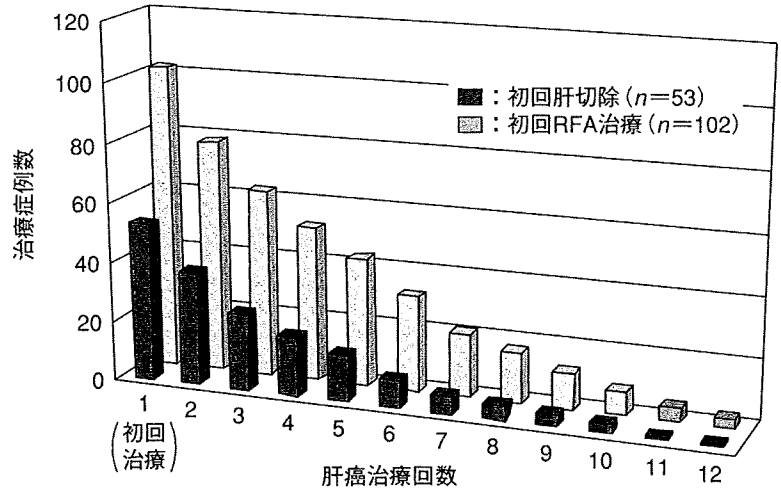

図 2　初回治療法別にみた肝癌治療回数

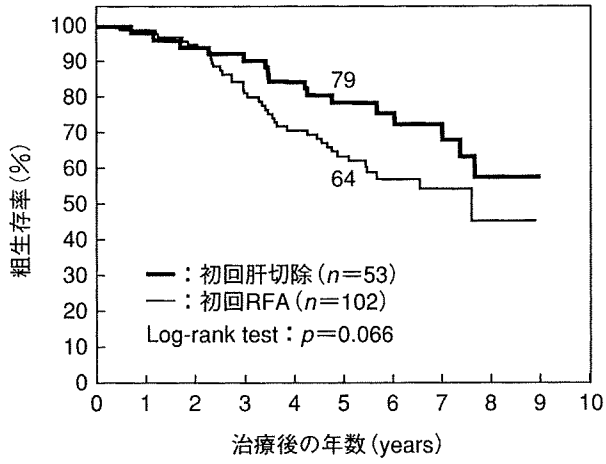

図 3　初回治療法別にみた肝癌治療後生存率

し再発に寄与する独立要因は，①初回治療法（RFA のハザード比 1.40, $p=0.0030$），②HBsAg（ハザード比 0.73, $p=0.021$），③年齢（65 歳以上のハザード比 0.81, $p=0.031$）の 3 要因があげられた．初回治療法が RFA であると，その後の繰返し再発の頻度が 1.4 倍になると計算されたが，初回再発リスク 1.76 より大きく低下した．

6. 初回治療法別にみた生存率

初回治療法別に累積粗生存率を求めた．肝切除群，RFA 群での 1 年生存率は 98.1%，99.0%，2 年は 94.3%，95.1%，3 年は 90.5%，81.2%，4 年は 84.7%，71.0%，5 年は 78.7%，63.8%で，肝切除群で生存率が高い傾向であった（log-rank テスト，$p=0.066$）（図 3）．生存率に寄与する独立要因を時間非依存性比例ハザードモデルで求めると，血小板数（10 万以上のハザード比 0.35, $p=0.002$），ICG 15 分値（31%以上のハザード比 1.70, $p=0.091$）で，これに初回治療法要因を強制投入しても，RFA でのハザード比は 1.05（$p=0.86$）と生存率には影響しなかった．

初回治療時の血小板数が生存予後に大きく影響するため，この比例ハザードモデル上で血小板数の平均値を代入して背景を標準化した仮想生存率曲線を作成した．これで初回治療法別に生存率を求めると，肝切除の 5 年生存率は 75.5%，RFA 群では 73.5%で，5 年生存率の差は 2%のみであった．

7. 考察

肝癌の経皮的局所治療は肝硬変合併症例でも施行可能で，肝予備能に対する影響も少ないとされ，RFA は小型肝癌の治療としてわが国では頻繁に行われている．しかし，外科的肝切除に比べて局所再発がわずかながら高いことが指摘されており，その後の再発予後・生存予後に影響することが懸念されている．今回の研究では，小型肝癌小型初回肝切除を選択するか RFA を選択するかが，その後の反復再発・QOL および生存予後にどれだけ影響するかについて検討した．

その結果，局所再発が若干存在することを反映し，初回再発率およびこれを通算した反復再発率の観点からは，RFA 治療で開始した症例で再発が多いことが明らかとなった．このことを受けて総治療回数・年間治療回数は RFA 治療群で多い傾向となったが，これに要する入院日数はやや少な

かった．再発の回数は多いものの，入院に必要な日数が少ないことはQOLおよび医療経済的には重要な事実と考えられた．

さらに，最終的な生存率の検討では肝切除群・RFA群は背景肝病変の差が大きく影響しており，この背景を補正すると初回治療がいずれであっても同様の生存期間が得られることが判明した．このことは，初回発癌率・再発発癌に関してはRFA治療群で高率であったが，“局所再発は追加治療でコントロールできる”ことを示唆しており，最終的な生存期間には影響しないことを意味した．すなわち，今後は患者のライフスタイルや治療に対する嗜好も考慮したうえで，QOL・医療経済的な観点での治療選択も必要であると考えられた．

国内外で行われたRFAと肝切除の比較

RFA治療初期の症例に行われたretrospectiveな分析で，Vivarelliら[1]は79例の肝切除症例と他院の79例のRFA治療症例を比較した．RFA群では在院日数が著明に短期間(肝切除：9日，RFA：1日)であったが，無再発生存率(3年無再発生存率，肝切除：50%，RFA：20%)・全生存率(3年生存率，肝切除：65%，RFA群：33%)の点では，肝切除が明らかに勝っていたとしている．また，翌2005年に発表された韓国からのretrospective study[2]でも，RFA 55例と肝切除93例の比較で，肝機能良好な小型肝癌ではRFA群での局所再発率が有意に高率であったとしている．しかし，異所再発率($p=0.30$)，全生存率($p=0.24$)，無再発生存率($p=0.54$)の点では有意差がなかったとしている．著者ら[3]も局所再発率の点で，RFA群は肝切除群より劣るが，局所再発に対する追加治療を考慮してもRFAは医療経済的に有利であることを示した．

中国のChenら[4]は直径5 cm以下単発の肝細胞癌180例を経皮的局所治療群と肝切除群に無作為に分け，この長期予後を検討した．経皮的治療群は19例が拒否したため最終的に71例，肝切除群は90例となった．1年・2年・3年・4年生存率は経皮的治療群で95.8%，82.1%，71.4%，67.9%，肝切除群では93.3%，82.3%，73.4%，64.0%で，両群はほぼ同様であった．また，1年・2年・3年・4年無再発生存率は経皮的治療群で85.0%，69.3%，64.1%，46.4%，肝切除群では86.6%，76.8%，69.0%，51.6%で，肝切除群がやや高かったが，有意差はなかった．術後合併症・侵襲などを考慮すると著者らは，経皮的治療群が手術よりも優れていると結論づけている．

最近では無作為化比較試験ではないが，イタリアのGuglielmiら[5]は200例の肝硬変合併肝細胞癌患者について，RFA治療109例と肝切除91例の予後を比較した．著者らの1施設での経験で肝癌は6 cm以下の症例である．生存期間の中央値は肝切除群57カ月に対してRFA群28カ月と有意に肝切除群で長かった($p=0.01$)が，Child分類B症例・多発肝癌症例では両群に差はなかった．3 cm以下の肝癌では無再発生存率・全生存率での差は認められなかったが，3 cmを超える症例では肝切除が多変量解析でも有意によい予後であったとして，わが国の一般的な考え方と同様な成績を示している．Abu-Hilalら[6]は外科医の立場から，2003年までの初期に行われたRFA症例との比較で，小型単発肝癌症例では無再発生存率の点で外科治療が優れているとしている．一方，Liangら[7]はretrospective研究で，肝切除とRFAでは無再発生存率・全生存率ともに差がなく，RFAは外科切除と同等であり，侵襲性からは外科治療より優れていると結論している．

現在，わが国では肝切除とRFAとの多施設無作為化試験が開始されており，長期予後の報告が期待されている．

おわりに

わが国の肝癌はウイルス性肝疾患，とくに肝硬変を基盤として発生することが多く，肝切除により“根治治療”しても5年で80%が再発をきたす．このように再発をきたすことを前提とした治療計画を立てることが肝癌診療の基本であり，このような立場から，個々の治療に侵襲性の低い経皮的局所治療が発達してきた．小型肝癌に対する治療選択は，再発リスク，生存予後予測，肝予備能，患者個人のQOL，医療経済などを考慮したうえでなされるべきある．著者らの成績では背景が同様の病態であれば，初回治療が肝切除かRFAかによ

り最終的な予後は影響されないと考えられた．今後は大規模な prospective study により長期予後の成績が出ることが期待されている．

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Occult hepatitis B virus infection increases hepatocellular carcinogenesis by eight times in patients with non-B, non-C liver cirrhosis: a cohort study

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SUMMARY. An impact of serum hepatitis B virus (HBV) DNA on hepatocarcinogenesis has not been investigated in a cohort of patients with non-B, non-C cirrhosis. Eighty-two consecutive Japanese patients with cirrhosis, who showed negative hepatitis B surface antigen and negative anti-hepatitis C virus, were observed for a median of 5.8 years. Hepatitis B virus core (HBc) region and HBx region were assayed with nested polymerase chain reaction. Both of HBc and HBx DNA were positive in 9 patients (11.0%) and both were negative in 73. Carcinogenesis rates in the whole patients were 13.5% at the end of the 5th year and 24.6% at the 10th year. The carcinogenesis rates in the patients with positive DNA group and negative DNA group were 27.0% and 11.8% at the end of the 5th year, and 100% and 17.6% at the 10th year, respectively ($P = 0.0078$). Multivariate analysis showed that men ($P = 0.04$), presence of HBc and HBx DNA (hazard ratio: 8.25, $P = 0.003$), less total alcohol intake ($P = 0.010$), older age ($P = 0.010$), and association of diabetes ($P = 0.005$) were independently associated with hepatocellular carcinogenesis. Existence of serum HBV DNA predicted a high hepatocellular carcinogenesis rate in a cohort of patients with non-B, non-C cirrhosis.

Keywords: hepatitis B virus, hepatocellular carcinogenesis, liver cirrhosis, occult hepatitis B virus infection, proportional hazard model.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a leading cause of death in many parts of sub-Saharan Africa and Asia [1,2]. It is also one of the most common neoplasms in Japan [3]. Hepatitis B virus (HBV) infection is the primary cause of cirrhosis and HCC and one of the major causes of death globally [4]. Needless to say, a cohort of patients with HBV-related chronic hepatitis and cirrhosis has a significantly high risk for HCC development [5–7]. In our retrospective cohort studies concerning HBV-related disease, cumulative hepatocellular carcinogenesis rates in chronic hepatitis ($n = 610$) and cirrhosis ($n = 180$) were 2.1% and 7.2% at the end of the 5th year, and 4.9% and 27.2% at the 10th year, respectively [5,7]. Abundant epidemiological and molecular biological evidence shows that HBV is an important factor in the development of HCC [8–10], but the precise role of HBV in the oncogenesis is still unknown.

HBV infection is usually diagnosed when the circulating hepatitis B surface antigen (HBsAg) is detected. However, the availability of highly sensitive molecular biology techniques has allowed the identification of HBV infection in HBsAg-negative individuals with or without circulating antibodies to HBsAg and/or hepatitis B core antigen (anti-HBc) [11–16]. Much evidence suggests that this so-called occult HBV infection is highly prevalent in a number of patient subgroups including those with HCV infection [16,17], cryptogenic advanced liver fibrosis [18] and HCC [17,19–27]. Although Marusawa *et al.* [28] and Uetake *et al.* [29] described the relationship between anti-HBc and HCC appearance rate in each study, impact of occult HBV infection on carcinogenesis cannot be evaluated because of lack of HBV DNA assay. As all the previous studies were performed as a pilot study or a case-controlled one, actual risk ratio of occult HBV infection for hepatocellular carcinogenesis has not been reported in a cohort study until now.

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartic transaminase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PCR, polymerase chain reaction.

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We, therefore, analysed a retrospective cohort of consecutive patients with cirrhosis for a long period, in order to elucidate the influence of occult HBV infection on the carcinogenesis rate from non-B, non-C cirrhosis.

PATIENTS AND METHODS

Patients

Among 103 consecutive patients diagnosed as having non-B, non-C cirrhosis by peritoneoscopic liver biopsy at Toranomon Hospital, Tokyo, Japan in the period from 1976 to 1998, initial frozen sera at the time of the diagnosis of cirrhosis were available for the assay of HBV DNA in 82 patients (79.6%). The cohort of 82 patients was retrospectively observed for a long period. All the patients showed negative HBsAg, negative anti-hepatitis C virus (HCV) and negative HCV RNA. Patients with a possible association of HCC at the time of the diagnosis of cirrhosis were strictly excluded from this study. No patient received interferon or other antiviral therapy after the diagnosis of cirrhosis.

Background and laboratory data of the patients

There were 67 men and 15 women aged 34–80 with a median age of 58 years. A total of 47 patients (57.3%) had a history of alcohol intake of more than 500 kg until the diagnosis of liver cirrhosis. Fifteen patients (18.3%) had decompensated cirrhosis with ascites, a history of encephalopathy, or both. The median value of indocyanine green retention rate at 15 min (ICG R15) was 33% (range, 7–75%), and total bilirubin concentration was 1.3 mg/dL (range 0.4–20.9 mg/dL).

Measurement of hepatitis virus markers

Hepatitis virus markers were assayed using frozen sera at −80 °C. All sera were tested for HBsAg (radioimmunoassay, Dainabot, Tokyo, Japan), anti-HCV (second-generation anti-HCV, enzyme-linked immunosorbent assay, Dainabot), and HCV RNA with reverse transcription-nested polymerase chain reaction (PCR).

HBV DNA was analysed for the region of HBc and HBx by sensitive nested PCR according to Yotsuyanagi *et al.* [30]. Fifty microlitres of STE solution [100 mmol/L Tris–HCl (pH 8.0), 100 mmol/L NaCl, 2 mmol/L ethylenediaminetetraacetic acid (pH 8.0), and 0.2% sodium dodecyl sulphate] with 20 μg of proteinase K (Boehringer, Mannheim, Germany) were added to serum samples. Mixed samples were then incubated for 2 h at 55 °C. DNA was extracted twice with phenol/chloroform, once with chloroform, and precipitated with ethanol. The DNA pellet was dissolved in 25 μL of TE buffer [10 mmol/L Tris–HCl (pH 8.0) and 1 mmol/L ethylenediaminetetraacetic acid (pH 8.0)].

Prepared DNA was subjected to amplification using nested PCR technique. HBV DNA was amplified using two independent pairs of primers, with each primer complementary to sequences in the X or core region of the HBV genome [30]. Amplification was performed using a thermal cycler for a total of 40 cycles, with each cycle consisting of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, in 100 μL of reaction mixture containing 200 mmol/L of each dNTP, 1X PCR buffer [50 mmol/L KCl, 10 mmol/L Tris–HCl (pH 8.3), 1.5 mmol/L $MgCl_2$ and 0.001% (w/v) gelatine], and 2 units of Ampli-Taq polymerase (Perkin Elmer Cetus Corp., Norwalk, CT, USA). The PCR products were separated in a 2% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Dassel, Germany). The membrane was then probed with digoxigenin-labelled oligonucleotides, which hybridize specifically with the core or X gene. Results were considered valid only if the same results were obtained in at least two separate experiments.

We considered the cases with positivity in at least two different viral genomic regions as HBV DNA positive. Appropriate negative controls were included in each PCR. The limit of sensitivity of our nested PCR methods ranged from 10 to 1 genome equivalents/mL.

Follow-up of patients

Follow-up of the patients was made on a monthly or bimonthly basis after diagnosis of cirrhosis by monitoring alpha-fetoprotein (AFP) and other biochemical data. Imaging diagnosis was made at least once a year for each patient with CT or US. After 1988, in order to detect HCC earlier, imagings were done three or more times per year in a majority of patients.

No patient underwent interferon therapy after the diagnosis of cirrhosis, but some of the patients received an oral or intravenous administration of medicinal herbs during the follow-up period.

All patients were finally evaluated in November 2004. The cases lost to follow-up were 13 (15.9%). The median observation period of the total patients was 5.8 years with a range of 0.1–34.8 years.

Statistical analysis

Differences of background features and laboratory data between the patients with and without HBV DNA were analysed by chi-square test, Fisher's exact test and Mann–Whitney's *U*-test. The time between diagnosis of cirrhosis and appearance of HCC was analysed using the Kaplan–Meier technique [31] and differences in curves were tested using log-rank test [32]. Those patients who had been lost to follow-up were regarded as censored data at the time of missing in the statistics. Independent risk factors associated with the appearance rate of HCC were studied using the stepwise Cox regression analysis [33]. Potential risk factors

assessed for hepatocellular carcinogenesis included the following 18 variables: age, sex, association of diabetes mellitus, total alcohol intake, history of cigarette smoking, family history of liver disease, history of blood transfusion, state of cirrhosis (presence of ascites and/or a history of encephalopathy), HBc DNA, HBx DNA, aspartic transaminase (AST), alanine transaminase (ALT), albumin, bilirubin, globulin, AFP, platelet, and ICG R15. A probability less than 0.05 was considered as significant. Data analysis was performed using computer program SPSS version 11 [34].

RESULTS

HCC appearance rate in all the patients

During the observation period, HCC appeared in 16 patients (19.5%). Median interval between the diagnosis of cirrhosis and HCC was 5.6 years (range 0.7–15.6 years) in the patients with HCC development. The cumulative HCC appearance rate in the 82 patients was 13.5% at the end of the fifth year after the diagnosis of cirrhosis, 24.6% at the end of tenth year, 33.3% at the 15th year, and 41.6% at the end of 20th year.

HCC appearance rates according to serum HBV DNA

Among the 82 patients, 9 patients (11.0%) showed positive serum HBV DNA and 73 (89.0%) negative HBV DNA. The former 9 patients had both HBc DNA and HBx DNA, and the latter 73 had neither of them. Table 1 summarizes the profiles and laboratory data of each group. There was no demographic difference between the two groups. There was also no statistically significant difference between them except for ALT value, which was lower in the patient group with positive HBV DNA (P = 0.028).

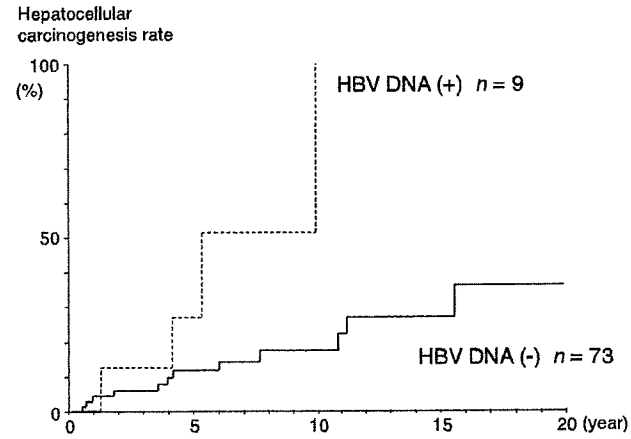

Fig. 1 Hepatocellular carcinogenesis curves of the patients with and without serum hepatitis B virus DNA. Carcinogenesis rates were 12.5% and 6.0% at the end of the third year, 27.0% and 11.8% at the fifth year, and 100% and 17.6% at the tenth year, respectively.

Figure 1 shows the curves of crude HCC appearance rate in the two patients group with and without serum HBV DNA. The third-year HCC appearance rates in the patients with and without DNA were 12.5% and 6.0%, the 5th-yr rates 27.0%, 11.8%, the tenth-yr rates 100% and 17.6%, respectively. The HCC appearance rate of the patient group

Table 1 Demography and laboratory data of patients with and without serum hepatitis B virus DNA

	HBV DNA*		
	Positive (n = 9)	Negative (n = 73)	P
Demographic and background features			
Sex – men/women	8/1	59/14	0.55
Age (median, range)	51 (45–68)	58 (34–80)	0.44
History of transfusion	1 (11.1%)	14 (19.4%)	0.55
Alcohol intake of 500 kg or more	5 (55.6%)	42 (58.3%)	0.87
Diabetes mellitus	3 (33.3%)	15 (20.8%)	0.40
Observation period (years)	5.7 (1.0–21.0)	6.1 (0.1–34.8)	0.92
Laboratory data (median, range)			
ICG R15 (%)	34 (12–51)	32.5 (7–75)	0.78
AST (IU/L)	32 (17–86)	40.5 (14–184)	0.26
ALT (IU/L)	16 (9–43)	28.5 (4–160)	0.028
Albumin (g/dL)	3.8 (2.6–4.5)	3.6 (1.7–5.2)	0.20
Bilirubin (mg/dL)	0.9 (0.5–2.8)	1.3 (0.4–20.9)	0.14
Platelet ($\times 1000/mm^3$)	142 (67–232)	104 (27–647)	0.18
AFP (ng/mL)	5 (3–9)	6 (1–98)	0.38

ICG R15, indocyanine green retention rate at 15 min; AST, aspartic transaminase; ALT, alanine transaminase; AFP, alpha-fetoprotein. *HBV DNA was assessed for HBc and HBx DNA using polymerase chain reaction

of positive HBV DNA was slightly higher than that of negative DNA (P = 0.0078, log-rank test).

Significance of serum HBV DNA in hepatocellular carcinogenesis

Cox proportional hazard model was performed for analysis of risk factors for liver carcinogenesis, using the 18 variables as mentioned above.

In the last step of stepwise regression analysis, the following five variables entered the model and could not be removed: sex (P = 0.005), serum HBV DNA (P = 0.003), past history of alcohol intake (P = 0.003), age (P = 0.035), and association of diabetes mellitus (P = 0.022) (Table 2). Accordingly, these five factors were significantly associated with hepatocellular carcinogenesis in the patients with non-B, non-C cirrhosis. Among them, gender was the strongest predictor of future HCC occurrence rate, indicating that male patients had 15.4 times as high carcinogenesis hazard as women patients. Similarly, positive HBV DNA (hazard ratio, 8.25) and little alcohol consumption of less than 500 kg (hazard ratio, 7.19) were the second and third strongest predictors for carcinogenesis, respectively. When the background factors of the cases were adjusted with the other significant factors, positive test for HBV DNA was significantly associated with the hepatocellular carcinogenesis rate.

Curves of carcinogenesis rates were generated from the multivariate analysis in an imaginary positive DNA group and an imaginary negative DNA group, with average sex ratio, average alcohol intake, average age and average association rate of diabetes (Fig. 2). The difference of the carcinogenesis curves indicated 'pure' impact of positive serum HBV DNA upon the carcinogenesis, which was adjusted with significant covariates assuming a standardized study group.

Table 2 Independent factors associated with liver carcinogenesis in the patients with non-B, non-C cirrhosis

Factors	Category	Hazard ratio (95% confidence interval)	P
Sex	Women	1	
	Men	15.4 (2.24–111.1)	0.005
Serum HBV DNA*	Negative	1	
	Positive	8.25 (2.01–33.93)	0.003
Total alcohol intake	≥500 kg	1	
	<500 kg	7.19 (1.98–26.32)	0.003
Age	<60 years	1	
	≥60 years	3.98 (1.10–14.42)	0.035
Diabetes mellitus	No	1	
	Yes	3.89 (1.22–12.47)	0.022

*Positive HBV DNA: positive for both HBc DNA and HBx DNA.

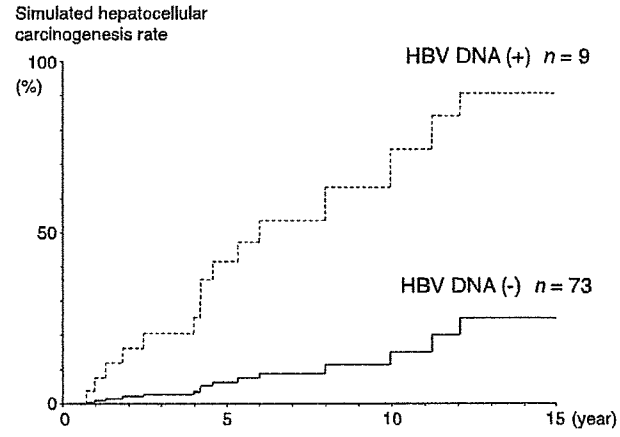

Fig. 2 'Adjusted' hepatocellular carcinogenesis rates in the positive HBV DNA group and the negative DNA group. Cox proportional hazard analysis showed that the carcinogenesis rate in the positive DNA group was significantly higher than that of the negative DNA group, when the other significant covariates were substituted with the same average parameters in the two groups.

Mortality and causes of death

During the observation period, 36 (43.9%) of 82 patients died: 5 (55.6%) of 9 patients in the positive DNA group and 31 (42.5%) of 73 patients in the negative DNA group. Cumulative survival rates in patients with and without HBV DNA were 78.8% and 74.1% at the end of the fifth year, 54.4% and 44.4% at the tenth year, 38.4% and 29.6% at the 15th year, and 33.6% and 29.6% at the 20th year, respectively. Although the survival rate in the positive HBV DNA group was lower than in the negative group, statistical significance was not shown.

Causes of death included liver failure due to liver cirrhosis in 21 (4 in positive DNA group and 17 in negative DNA group), progression of HCC in 7 patients (all in negative DNA group), and other causes in 8 (one in positive DNA group and 7 in negative DNA group).

DISCUSSION

Epidemiological and molecular virological studies in the 1970s and early 1980s established a strong aetiological association between chronic HBV infection and the hepatocellular carcinogenesis [35]. We also estimated annual carcinogenesis rates as 0.5% in chronic hepatitis and 3% in cirrhosis, from cohorts of biopsy-proven HBV disease [5,7].

Integration of HBV DNA has been reported in the majority of HBsAg positive HCCs since 1980s, and the fact suggested HBV might be oncogenic. Up to now, there is no evidence

that HBV DNA is directly oncogenic and the mechanism by which chronic HBV infection leads to carcinogenesis remains unclear. Integration of HBV DNA may stimulate cellular pro-oncogenes or suppress growth-regulating genes [36]. Integration of HBV DNA, however, has been found in varied regions of the host chromosomes and no preferential and specific site has been identified until now. The other authors suggested that integration of HBV DNA could also induce carcinogenesis via transactivation of other oncogenes [37]. Both HBx protein and the truncated pre-S/S protein are potent transactivators and are commonly found in HCC tissue but their precise role in hepatocarcinogenesis remains unknown.

Occult HBV infection is generally defined as the detection of HBV DNA in the serum or liver tissue of patients who test negative for hepatitis B surface antigen [38–41]. Occult HBV infection was first reported in the early 1980s when hybridization techniques for the detection of HBV DNA became available. These studies showed that HBV DNA could be detected in HBsAg negative patients with HCC [42]. Recent studies using more sensitive techniques confirmed the close correlation between chronic occult HBV infection and carcinogenesis. Many authors demonstrated the relationship between occult HBV infection and hepatocellular carcinogenesis, mainly by a pilot study or a case–control study [17,19–27]. Shiota *et al.* [24] reported in their case studies without control group that serum of 18 out of 26 HCC patients without HBsAg and anti-HCV were positive for either S, C, or X region on PCR and southern blotting. Pollicino *et al.* [26] described that viral DNA was detected in 68 of 107 cases of HCC tissue (63.5%) and in 63 of 192 cases of chronic hepatitis tissue (32.8%), and concluded that occult HBV is a risk factor for development of HCC. The other authors also emphasized the high incidence of HBV DNA in either serum or HCC tissue compared with that of cases without HCC development. All the literatures, except one [43] from Taipei where HBV infection was endemic and prevalent, concluded that occult HBV infection was closely associated HCC development. However, precise risk or hazard ratio for carcinogenesis has not been reported.

Current study on this topic provided strong evidence of an association between occult HBV infection and HCC. In the patient cohort of non-B, non-C cirrhosis, occult HBV infection increased the future carcinogenesis rate with a hazard ratio of 8.25 (95% confidence interval, 2.01–33.93). It has been proposed that diagnosis of occult HBV infection be made only when HBV DNA can be detected using at least two sets of primers from different areas of the HBV genome in duplicate assay [38,39]. Appropriate negative controls must be included in each assay and specificity of the amplification reaction confirmed by sequencing of the amplicons. Using this strict criterion, occult HBV infection was found in 9 (11.0%) of 82 Japanese patients with non-B, non-C cirrhosis. Background features of the nine patients with serum HBV DNA showed a slightly younger age, a lower ALT, a slightly lower bilirubin, and a slightly higher platelet count (Table 1). Although all these demographic and laboratory findings were considered to favour low carcinogenetic risk, the patients with cryptic HBV DNA infection developed HCC more frequently. After adjustment of these background covariates in the multivariate analysis, positivity of serum HBV DNA proved to be an independent risk factor for hepatocarcinogenesis (Table 2).

As this retrospective cohort consisted of only cirrhosis as an advanced liver disease, and as it included both alcoholic and non-alcoholic cirrhosis, the hazard ratio of 8.25 could not be applied for varied stages and varied aetiologies of liver disease. In order to elucidate the impact of occult HBV infection on carcinogenesis, future studies should be performed also in the other cohort of chronic liver disease, such as HCV-related disease. Although anti-HBc and anti-HBs antibody were measured in a small numbers of the patients, an exact relationship between serum HBV DNA and serum positivity of anti-HBc antibody was not analysed in this study. When we tested anti-HBc antibody in a small part of subjects, 3 of 6 patients (50.0%) with positive HBV DNA had serum anti-HBc antibody and 7 of 19 patients (36.8%) without HBV DNA had anti-HBc (Fisher's exact test, $P = 0.69$). For the convenience of clinical circumstance and practical usefulness, significance of positive anti-HBc on carcinogenesis risk should be elucidated through a large-scale cohort study with an identical assay for anti-HBc antibody.

Although a lot of epidemiological and clinicopathological evidence of the relationship has been published, precise role of occult HBV in this setting has been still unclear. Patients with occult hepatitis B overlap with those who previously have been classified as having recovered [44]. In fact, the distinction between recovery and occult hepatitis B is likely to be somewhat arbitrary, as recovery does not necessarily imply eradication of infection in all cases [30], but includes the possibility of complete suppression in some cases by a broad and vigorous immune response [44]. One of the most important clinical questions is whether occult hepatitis B merely represents a marker of past infection, or whether HBV genome persistence contributes to liver disease. It is very likely that occult HBV is a cofactor in the development of HCC. Several studies found that patients co-infected with HBV and HCV have increased risks of HCC compared with those with mono-infection. Our cohort studies [45] also showed that a risk factor of a history of heavy drinking interacted with HBV or HCV subtypes in a characteristic manner from the viewpoint of carcinogenesis in cirrhosis. The other important problem is whether occult HBV infection alone causes HCC. To address this question, studies on occult HBV infection in patients with HCC might provide details on other causes of chronic liver disease including nonalcoholic fatty liver disease, which may masquerade as cryptogenic cirrhosis, hemochromatosis, alfa-1-antitrypsin deficiency, and autoimmune liver disease [46]. Recently,

Castillo *et al.* [47] reported a clinical state of occult HCV infection, which shows negative serum anti-HCV, negative serum HCV RNA, and positive HCV RNA in liver biopsy specimen. Although we did not tested the possibility of occult HCV infection in this study, future studies should be also aimed at the influence of latent HCV infection on hepatocarcinogenesis.

In conclusion, occult HBV infection significantly increased the incidence of hepatocellular carcinogenesis in patients with non-B, non-C cirrhosis. Although non-B, non-C cirrhosis seemed to include varied aetiology of liver disease, cryptic HBV infection should be taken account in the prediction of future HCC development.

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Original Article

Efficacy of platinum analogue for advanced hepatocellular carcinoma unresponsive to transcatheter arterial chemoembolization with epirubicin

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Aim: Hepatocellular carcinoma (HCC) often shows resistance to transcatheter arterial chemoembolization (TACE). Such patients often have a poor prognosis and are unresponsive to other forms of therapy. The aim of this retrospective study was to determine the response to TACE using platinum analogues in patients deemed resistant to TACE using epirubicin.

Methods: We studied 152 consecutive patients with advanced HCC resistant to TACE using epirubicin. All cases were treated with platinum analogue using transcatheter arterial chemotherapy with or without embolization.

Results: Computed tomography at 3 months after therapy showed complete response (CR) in 6 patients (4.0%), partial response (PR) in 28 (18%), stable disease (SD) in 35 (23%), and progressive disease (PD) in 83 (55%). The cumulative survival rates for PR/CR patients who received platinum analogue-transcatheter arterial chemotherapy with or without embolization (81.8% at first year, 53.9% at second year, and 33.1% at third year) were significantly higher than those of SD/PD patients (36.6%, 17.5% and 7.4%, respectively) ($P < 0.001$). The 50% survival period was extended almost 1.4 year in PR/CR patients who received platinum analogue-transcatheter arterial chemotherapy with or without embolization.

Conclusion: Our retrospective study is the first to report the efficacy of platinum analogues for advanced HCC unresponsive to TACE using epirubicin.

Key words: hepatocellular carcinoma, platinum analogue, transcatheter arterial chemoembolization, unresponsive

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common neoplasms in Africa and Asia including Japan. Since it is a well known that more than 80% of the cases with HCC are associated with liver cirrhosis, a routine check-up for cirrhotic patients with ultrasound (US) could potentially lead to the detection of small HCC. However, because of the association of cirrhosis and tumor multiplicity, surgical resection is performed only in 20% of the cases or less.[1,2] Transcatheter arterial chemoembolization (TACE) has been reported to be an effective palliative treatment for patients with unresectable HCC, and many chemotherapeutic agents such as doxorubicin, epirubicin, mitomycin were used with lipiodol in Japan.[3–10] Although repeated TACE is one of the most potent therapies for unresectable HCC, resistance to the therapy often ensues after therapy repetition, and long-term survival rates after 3 years are not sufficiently high at present.

Platinum analogues are effective against many malignant tumors, and in recent years, they have been used in the treatment of HCC. It has been reported that carboplatin-lipiodol treatment improved 1-year survival rate compared with doxorubicin-lipiodol treatment in patients with advanced HCC.[11] As for cisplatin, several studies reported its effectiveness for advanced HCC. Furthermore, the efficacy of cisplatin and lipiodol combination therapy has been reported by several investigators.[12–19] To our knowledge, however, there is no information on the efficacy of platinum analogues in TACE- epirubicin resistant HCC patients.

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The purpose of this retrospective study was to examine the efficacy of platinum analogues (carboplatin and cisplatin) for advanced HCC unresponsive to TACE using epirubicin.

PATIENTS AND METHODS

Study population

FROM 1980 TO 2006, 1,250 patients were diagnosed with HCC at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these, 565 patients underwent TACE treatment for HCC. Among the 565 patients, 184 patients were judged by two hepatologists as embolization-resistant HCC and they received a platinum analogue. All 184 patients had been considered to have unresectable HCC at the time of diagnosis of HCC, and had undergone TACE therapy at least twice until being considered TACE-resistant. Embolization-resistant HCC was defined as an HCC whose number and/or size had increased in the treated segment and/or extended other segments despite repetitive course of TACE using epirubicin, lipiodol and gelatin sponge. Thus, this retrospective cohort study was based on 184 consecutive patients with TACE-resistant HCC.

Before treatment with carboplatin or cisplatin, all the patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver imaging studies [computerized tomography (CT), ultrasonography (US), digital subtraction angiography (DSA)], complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of US, CT, and DSA.

Of the 184 patients, 32 were excluded because they did not meet the following inclusion criteria: (i) typical hypervascular HCC by all imaging modalities; (ii) no history of other malignancies; (iii) no evidence of extrahepatic metastasis of HCC; (iv) performance status of 0–1; (v) adequate liver function with bilirubin value of 5 mg/dL or less; (vi) sufficient hematopoietic function with a platelet count of more than 25,000/mm^3 and leukocyte count of more than 2,000/mm^3; and (vii) an expected survival time of at least 3 months. All patients gave informed consent to the treatment. Accordingly, 152 patients with TACE-resistant HCC were retrospectively evaluated for efficacy of platinum analogue for advanced HCC unresponsive to TACE-epirubicin. The observation starting point was the time of first therapy using platinum analogue at our hospital.

Serologic markers for HCV and HBV

The diagnosis of HCV infection was based on detection of serum HCV antibody with RNA positivity. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc., NJ). Hepatitis B surface antigen (HBs-Ag) was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). Serum HBV-DNA level was determined independently, using the nested PCR, by an experienced technician (M.K), who was blinded to the clinical information. The used serum samples were stored −80°C at first consultation.

Treatment protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and a catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the platinum analogue with or without Lipiodol (Lipiodol Ultrafluide, Laboratoire Guerbet, Aulnay-sous-Bois, France) and small gelatin cubes (1 × 1 mm). The platinum analogue used was either carboplatin at 150 to 450 mg/body (63% of patients received 450 mg/body) or cisplatin at 40 to 100 mg/ body of (36% of patients received 100 mg/ body). Both analogues were administrated slowly under careful fluoroscopic guidance. When using Lipiodol, the platinum analogue and Lipiodol were divided into six to eight parts and mutually injected. In patients who received Lipiodol, the volume of injected Lipiodol ranged from 2.0 to 5.0 mL. The dose of Lipiodol was determined according to tumor size and the degree of liver dysfunction.

Selection criteria of type of therapy

Patients were treated by three type of therapy, depending on the extent of their tumors and liver function; (i)hepatic arterial injection (HAI) were performed for those patients with a tumor thrombus into main portal trunk or with severe liver function, (ii)chemolipiodalizatio (CL) were performed for those patients with tumor thrombus in distal portal branch complicated with severe liver function, (iii)TACE were performed for those patients without main portal vein thrombus and severe liver function.

Background and laboratory data

Table 1 summarizes the profiles and data of 152 patients who were treated with carboplatin or cisplatin. The

Table 1 Demographics and laboratory data of 152 patients with HCC who underwent transcatheter arterial chemotherapy using platinum analogue for advanced HCC unresponsive to TACE-epirubicin

Parameter	(n = 152)
Patient characteristics	
Sex (M : F)	122:30
Age (years)	67 (38–85)
Back grounds of liver disease	
Hepatitis B surface antigen positive	27
HCV antibody positive	123
Both negative	2
Status of liver function	
Child-Pugh classification (A/B/C)	98/51/3
Laboratory data	
Platelet count ($\times 10^4$ /μL)*	11.0 (3.4–35.5)
Albumin (g/dL)*	3.0 (2.1–4.5)
Bilirubin (mg/dL)*	1.0 (0.3–4.3)
AST (IU/L)*	68 (21–488)
Prothrombin time (%)*	84 (45.5–114)
ICG R15 (%)*	38 (6.0–76)
AFP (μg/L)*	236 (2.0–112,000)
DCP (AU/L)*	153 (10–131,000)

*Expressed as median (minimum, maximum).
AFP, alpha-fetoprotein; AST, aspartate aminotransferase; DCP, des-gamma carboxyprothrombin; HCC, hepatocellular carcinoma; ICG R15, indocyanine green retention rate at 15 minutes; TACE, transcatheter arterial chemoembolization.

patients consisted of 122 men and 30 women, and their age ranged from 38 to 85 years (median, 67 years). They included 27 (18%) HBs-Ag positive patients, 123 (81%) HCV antibody positive patients, and 2 (1%) negative for both. At the time of the first platinum analogue treatment, the median serum albumin concentration was 3.0 g/dL, total bilirubin 1.0 mg/dL, indocyanine green retention rate at 15 minutes (ICG R15) 38%, prothrombin activity 84%, alpha-fetoprotein (AFP) 236 μg/L, and des-gamma-carboxyprothrombin (DCP) was 153 AU/L. As for Child-Pugh classification, 98 (64%) were class A, 51 (34%) were class B, and 3 (2%) were class C patients.

Characteristics of hepatocellular carcinoma

Table 2 summarizes the profiles of HCC that were treated with platinum analogue. The median tumor size was 40 mm. A solitary HCC was detected in 6 (4%) patients while multiple HCC were detected in 146 patients at the time of the first platinum analogue treatment. For the latter group, the tumors were localized to one segment in 10 (7%) patients, to one lobe in 32 (21%) patients, and in both lobes in 104 (68%) patients. Portal vein invasion was noted in 46 (30%) patients. The number of courses of TACE-epirubicin until judgment of embolization-resistance ranged from 2 to 16 with a median of 4 courses. The median interval between diagnosis of HCC and judgment of embolization-resistance was 30.1 months.

Table 2 Profile of HCC in 152 patients who underwent transcatheter arterial chemotherapy using a platinum analogue for advanced HCC unresponsive to TACE-epirubicin

Profiles of liver cancer	
Tumor size (mm)*	40 (8–180)
Intrahepatic multiplicity	
Solitary	6
Multiple, localized to one segment	10
Multiple, localized to one lobe	32
Multiple, extended to both lobes	104
Portal vein invasion (no/yes)	106/46
Embolization iteration until unresponsiveness	4 (2–16)
The kind of used platinum analogue	
Carboplatin/Cisplatin	105/47
Treatment method	
HAI/CL/TACE	73/20/59

*Expressed as median (minimum, maximum).
CL, chemolipiodalization; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

The type of platinum analogue used for treatment was carboplatin for 105 (69%) patients, and cisplatin for 47 (31%) patients. With regard to the method used for delivery of platinum analogue, hepatic arterial injection (HAI) was used in 73 (48%) patients, chemolipiodalization (CL) was used in 20 (13%) patients and TACE in 59 (39%) patients.

Assessment of therapeutic effects and follow-up

The effects of chemotherapy were evaluated by CT every three months after treatment. The presence of non-enhanced tumor areas reflects tissue necrosis, and according to the findings of this imaging technique, the response to treatment was defined according to the World Health Organization criteria:[20] complete response: no evidence of neoplastic disease; partial response: reduction in total tumor load of ≥ 50%; no change: reduction of < 50% or increase of < 25%; progressive disease: increase of ≥ 25%.

Patients were examined by physicians every 4 weeks including monitoring of AFP, DCP and other biochemical data after the diagnosis of embolization-resistance.

Table 3 Profile of 152 HCC patients who underwent transcatheter arterial chemotherapy using a platinum analogue for advanced HCC unresponsive to TACE-epirubicin, according to type of therapy

	HAI ($n = 73$)	CL ($n = 20$)	TACE ($n = 59$)
Profile of liver cancer and tumor marker			
Tumor size (mm)*	48 (8–180)	40 (8–100)	33 (12–180)
Intrahepatic multiplicity			
Solitary	4	1	1
Multiple, localized to one segment	3	2	5
Multiple, localized to one lobe	14	7	11
Multiple, extended to both lobes	52	10	42
Portal vein invasion (no/yes)	45/28	12/8	49/10
AFP (μg/L)*	257 (3–112000)	682 (17–65900)	145 (2–103000)
DCP (AU/L)*	400 (10–131000)	68 (10–53900)	40 (10–55420)
Status of liver function			
Child-Pugh classification (A/B/C)	43/28/2	13/6/1	42/17/0

*Expressed as median (minimum, maximum).
AFP, alpha-fetoprotein; CL, chemolipiodalization; DCP, des-gamma carboxyprothrombin; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

Imaging studies, as required for measurement of tumor size, were performed at around 3 months after therapy. Some patients took oral or intravenous medicinal herbs or other palliative therapies during the follow-up period.

Statistical analysis and ethical considerations

The factors that influenced the treatment outcome ((partial response (PR) or complete response (CR)) in this cohort were analyzed by the χ^2 test, and the cumulative survival rate was analyzed by Kaplan-Meier method. The risk factors involved in survival were evaluated by univariate analysis with the log-rank test. The independent factors associated with the curative effect (PR or CR) and survival rate were identified using the stepwise Cox regression analysis. Potential risk factors assessed for curative outcome (PR and CR) and survival rate included the following 17 variables: age, sex, HBs-Ag, HCV-antibody, aspartate transaminase (AST), albumin, bilirubin, AFP, DCP, prothrombin activity, ICG-R15, tumor size, multiplicity, portal vein invasion of HCC, treatment methods (HAI/CL/TACE), the type of platinum analogue (carboplatin/cisplatin) and the dose of platinum analogue used for treatment. Several variables were transformed into categorical data consisting of two-three simple ordinal numbers for univariate and multivariate analyses. All factors found to be at least marginally associated with the curative effect and survival ($P < 0.10$) in univariate analysis were entered into a multivariate logistic regression and Cox proportional hazard models. Significant variables were selected by stepwise method in the procedure. Proportional hazard analysis was also employed in the identification of contributing factors to the curative effect and survival rate. A P-value of less than 0.05 in two-tailed test was considered significant. Data analysis was performed using SPSS software version 11.0 (SPSS Inc, Chicago, Ill).

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital. The physicians in charge explained the purpose and method of the clinical trial to each patient, who gave their informed consents for participation.

RESULTS

Efficacy of platinum analogue, according to type of therapy

TABLE 3 SUMMARIZES the profiles and data of 152 HCC patients who were treated with platinum analogue, according to type of therapy.

In these patients, 6 of 152 (4%) patients showed CR, 28 (18%) patients showed PR, 35 (23%) patients showed stable disease (SD), and 83 (55%) patients showed progressive disease (PD). Analysis according to type of therapy showed 73 of 152 (48%) patients received HAI, 20 (13%) received CL, and 59 (39%) received TACE. The efficacy of transcatheter arterial chemotherapy using platinum analogue according to the type of therapy was as follow; in HAI group: 1 of 73