れる．B型慢性肝疾患での肝癌発癌には，肝病変進展度が高度な症例ほど（肝硬変），HBVDNA濃度が高いほど発癌率が高いことが広く知られており，核酸アナログなどによってウイルス量を低下させることで発癌抑制・肝病変進行抑制につながる．

C型肝硬変に対するIFNの効果はやや低い．C型慢性肝疾患では，AFPやALTが低下した状態では肝癌発癌率が低下するとの成績も報告されているが，ALTがどのレベルまで低下すればよいのかなど，具体的な指標はまだ明らかになってはいない．今後は，発癌高危険群かつ肝不全高危険群との観点で，発癌予防・肝硬変進行予防の観点で，SVR以外の治療目標や最適な治療スケジュールを明らかにしていく必要がある．

おわりに

本稿は，肝硬変に対する「抗ウイルス療法」の内容であるが，インターフェロン治療以外でもトランスアミナーゼを安定化させることによる肝病変進展予防・発癌予防，さらに肝硬変病態に対する栄養補助療法など，あらゆる側面から肝疾患進行・肝癌発癌を予防する手段を柔軟に駆使することこそが重要である．

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特集・肝炎のインターフェロン治療の最前線

［C型肝炎］症例別にみたインターフェロン治療の実際

C型肝硬変・肝癌治療後の患者に対するインターフェロン治療

池田健次*

Summary

C型肝硬変・肝癌に対するインターフェロン治療でのウイルス排除（SVR）率は慢性肝炎より劣る。またSVRとなっても発癌抑制効果は慢性肝炎の場合より不良で，慢性肝炎例で10分の1であるのに対し，肝硬変では2分の1に減少する程度である。肝硬変診断例・肝癌根治治療後症例は，高齢・肝機能不良・合併疾患などを理由にSVRを目指した強い抗ウイルス療法ができないことも多く，肝の壊死炎症反応抑制による発癌率低下を目指す長期少量インターフェロン治療も考慮すべきである。

Key Words　肝硬変／肝細胞癌／C型肝炎ウイルス／インターフェロン／発癌抑制

はじめに

近年は外来通院中のC型肝炎患者の高齢化が目立つ一方，診療している患者の重症化（線維化進行例の相対的増加）がみられる。当然，高齢者・線維化進行例に対するインターフェロン（IFN）治療例が増加し，副作用発生率や治療成績不良を考慮せねばならない。2000年以後も3万人を超す肝癌患者の死亡が続いており，生命予後に直結する肝癌再発を抑制するためのIFN治療も盛んに行われている。

2006年よりC型肝硬変に対して初めてIFNβの保険認可が得られ，2008年には肝硬変に対するIFNαの認可が続き，2010年4月からは肝炎対策基本法に基づく積極的なIFN治療の医療費助成が更新された。本項では，C型肝硬変患者・肝癌治療後の症例に対するIFN治療の実態・効果について述べる。

C型肝硬変に対するインターフェロン（IFN）治療

当院で診療したC型肝硬変885例の長期予後を，IFN治療の有無別に，retrospectiveに検討した。

1．対象・方法

対象は2005年までの間に虎の門病院肝臓科に入院した肝硬変症例のうち，HBsAg陰性・C型肝炎ウイルス（HCV）抗体陽性の885例

＊虎の門病院肝臓内科部長

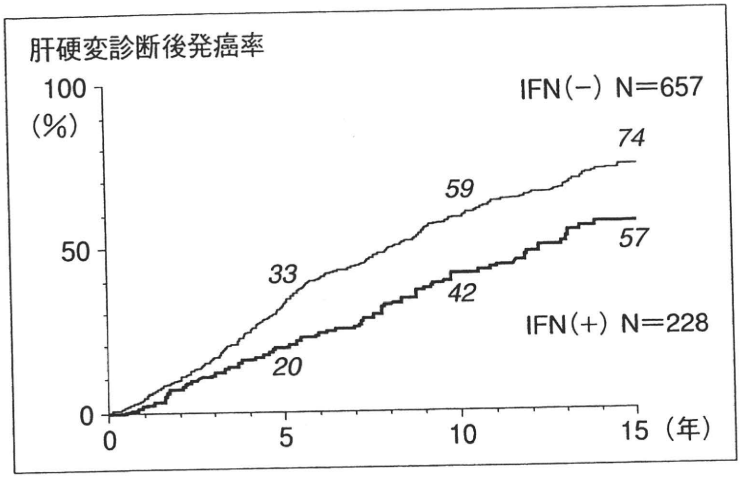

図1　インターフェロン投与有無別にみたC型肝硬変診断後肝癌発癌率

とした。肝硬変の診断は，腹腔鏡肝生検による診断527例と，臨床症状・超音波・内視鏡・判別計算式などによる臨床診断例358例とが含まれている。6ヵ月以上の観察期間が行われた例のみを対象とし，長期予後に関する検討を行った。

885例中228例（25.8%）にIFN投与を行った。IFN非投与の657例と投与228例の比較をみると，IFN投与群では男性の比率が高く，年齢は5.5歳若年であり，肝機能障害はわずかに軽度な傾向がみられた。

2．成　　績

1）IFN投与群での治療効果

228例に行われたIFN治療で，持続的なウイルス消失が得られた（SVR）のは53例（23.2%），持続的なウイルス消失が得られないものの治療後6ヵ月以上ALT正常化がみられた（BR）のは21例（9.2%），ウイルス・ALTともに効果の得られなかった（NR）のは111例（48.7%）で，残る43例（18.9%）は現在投与中・転院による観察不能・その他判定不能例であった。

このうち1b型高ウイルス量の難治性96症例では，SVR 8例（8.3%），BR 7例（7.3%），NR 56例（58.3%），その他25例（26.0%）であったが，2型か低ウイルスのいずれかであった非難治性116例では，SVR 43例（37.1%），BR 13例（11.2%），NR 40例（34.5%），その他12例（10.3%）であった。

2）肝硬変診断後の肝癌発癌率

IFN未治療の657例からは観察期間に369例（56.2%）の肝癌発癌がみられ，IFN治療施行228例からは96例（42.1%）に肝癌発生がみられた。

両群の肝癌粗発癌率を，肝硬変診断日を観察開始として計算すると（図1），IFN未治療群・治療群からの発癌率はそれぞれ，3年 16.7%・11.1%，5年 33.0%・19.9%，7年 44.3%・25.5%，10年 58.9%・42.1%，15年 74.2%・56.9%で，IFN投与群では有意に発癌率が低かった（log-rank test，$P<0.0001$）。

IFN投与群での肝癌発癌率はIFN投与開始日を起点として計算すると，IFN未治療群・治療群からの発癌率はそれぞれ，3年 16.7%・15.8%，5年 33.0%・26.8%，7年 44.3%・34.4%，10年 58.9%・47.3%，15年 74.2%・66.2%で，両群の差は縮まったものの，IFN投与群では有意に発癌率が低かった（log-rank test，$P=0.019$）。

3）肝癌発癌に寄与する要因

C型肝硬変からの発癌に寄与する独立要因をCox比例ハザードモデルで検討した。治療効果を高める要因は，男性，20 ng/mL以上のAFP高値，55歳以上の高年齢，10万/μL未満の血小板数，30%以上のICG 15分値が有意に肝癌発癌率を高めた。これらの独立要因を共変量として，IFN治療要因を時間依存性変数として投入したモデルでは，IFN投与

症例全体の発癌ハザード比は0.86と低かったが，統計学的に有意水準には達しなかった（P＝0.22）。

IFN治療効果をSVR・BR・NR・継続投与中の4群に分けて発癌寄与要因を検討した。性別・AFP値・年齢・血小板数の各共変量で補正した後のIFN治療効果別にみた肝癌発癌へのハザード比は，SVR＋BR例では0.35（P＜0.001），継続投与中0.47（P＜0.13）で，無治療群より発癌抑制傾向であった。

4）肝硬変診断後の粗生存率

IFN未治療の657例のうち観察期間に442例（67.3%）が死亡したが，IFN治療施行228例では64例（28.1%）が死亡したのみであった。

両群の粗生存率を，肝硬変診断日を観察開始として計算すると，IFN未治療群・治療群での累積生存率はそれぞれ，3年 92.3%・96.8%，5年 81.8%・94.9%，7年 68.9%・88.8%，10年 48.4%・88.8%，15年 25.0%・59.8%で，IFN投与群では有意に生存率が高かった（log-rank test，P＜0.0001）。

IFN投与群での累積生存率を，IFN投与開始日を起点として観察期間とする計算を行うと，IFN未治療群・治療群での生存率はそれぞれ，3年 92.3%・95.9%，5年 81.8%・91.4%，7年 68.9%・84.1%，10年 48.4%・73.7%，15年 25.0%・52.2%で，両群の差は縮まったものの，IFN投与群では有意に生存率が高かった（log-rank test，P＜0.0001）。

5）肝硬変診断後の生存率に寄与する要因

C型肝硬変症例の生存に寄与する独立要因をCox比例ハザードモデルで検討した。生存期間を短縮する要因は，3.9 g/dL以下の低アルブミン，55歳以上の高年齢，男性，30%以上のICG 15分値，20 ng/mL以上のAFP高値，IFN治療要因であった。IFN投与例中にはSVR・BR・NRの症例が含まれているが，IFN投与群では死亡ハザードが0.15に低下（P＝0.042）した。

3．評　価

肝硬変まで進行した症例のretrospectiveな分析であるが，多数例に対して長期の経過観察が行われている。IFNを投与した症例は26%と少なく，そのうちSVR率は23%にすぎなかった。無作為化比較試験ではないとの限界をふまえた上で，肝硬変からの発癌率・生存率に強い影響を及ぼすと考えられる共変量を4因子以上使用して多変量解析を行ったところ，IFN投与症例全体では肝細胞癌発癌に及ぼす効果は小さかった（ハザード比 0.86）。このうち，SVR・BRに至った症例での発癌抑制効果は明らかであった（ハザード比 0.35）。

肝癌発癌・再発に対するIFNαとIFNβの違い

慢性肝疾患を基盤として発生する肝細胞癌は，根治療法を施行した後も再発率が高いことが知られており，3年で約50%，5年で約80%の再発率に達するとされている。わが国ではC型慢性肝疾患に由来する肝癌が多く，肝機能不良とともに，高い再発率に対する対策が大きな問題点となっている。

再発率を抑制する手段として，我々は2000年にHCV関連肝癌根治療法後に無作為化比較試験でIFNβの再発抑制効果を検討した[1]。この少数例のprospective studyの成績では，肝癌再発率がIFNβで有意に抑制できることが示されたが，IFNβとIFNαでの肝癌発癌抑制効果・再発抑制効果の比較に関してはいくつかの相違が報告されている。Kashiwagi

ら[2]はIFN治療連続351例で治療効果と発癌率をIFNαとIFNβとで比較した。この結果，IFNαでの治療後には6.5%，IFNβの後には4.4%の発癌率がみられ，特にIFNβ治療後にはNR（ウイルス効果・生化学的効果ともになし）の群からの発癌率が低かったと述べている。Damdinsurenら[3]は肝癌cell lineで抗癌剤にIFNαとIFNβを併用した比較を述べている。彼らは，βの方が，使用したすべてのcell lineで細胞増殖抑制効果が高かったと述べており，抗癌剤併用IFN治療はβを用いた方が有利であると結論付けている。Matsumotoら[4]は，ヒト肝細胞癌細胞の増殖に及ぼす影響をIFNαとIFNβとで比較した。IFNα2bとβのいずれも細胞増殖は有意に変化させなかったが，α2bではERK1/2の活性化のみを示したのに対し，βではERK1/2とAKTの両者を活性化したとしている。Murataら[5]は，HepG2，Huh7，JHH4の3種のcell lineに対する増殖抑制効果・細胞周期の変化・アポトーシスなどをIFNαとIFNβとの間で比較した。この結果，IFNβの方が，増殖抑制効果，S期への停滞，アポトーシス誘導，HLA class I分子の表出，Interferon-stimulated gene（ISG）の表出などすべての点で強力であったとしている。Damdinsurenら[6]は3種のcell lineと異種移植マウスモデルの実験で，IFNβの方が*in vitro*でも*in vivo*でもより強い腫瘍細胞増殖抑制効果を示したことを示している。以上のように，IFNαとIFNβの間では，肝癌細胞に対する作用はβの方が強力であるとする報告が多い。

C型肝細胞癌根治療法後のIFNαによる再発抑制効果

わが国では慢性肝炎・肝硬変ともにα，βのIFNを使用することが可能であるが，日本以外の海外諸国では使用できないこと，静脈注射でありやや扱いが面倒であること，価格の点で不利であることなど，IFNβが広く使用されるには多くの隘路がみられる。以上のような背景をふまえ，ここではHCV関連肝癌に対して根治治療後にIFNαを使用した症例についてretrospectiveに検討した。

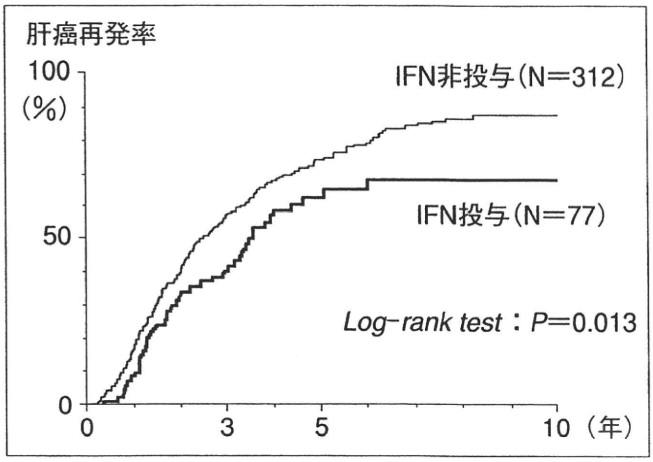

図2　インターフェロン投与有無別にみた根治療法後の肝癌再発率

1．対象・方法

対象は1990年～2007年の間に当科に入院し，治療を行った初発HCV関連小型肝癌389例とした。症例は，最大径3 cm以下・3個以内の肝癌で，外科切除または根治的ラジオ波凝固療法（RFA）を施行した症例とした。

この間にIFNα治療を行った症例は77例，非投与例は312例であった。IFNβ治療症例およびレチノイド治験参加症例は検討から除外した。IFN投与例と非投与例に分けて，背景因子を比較した。年齢の中央値はIFN投与群で63歳と非投与群より3歳若年であった（P＝0.003）。しかし，男女比は投与群 46：31，非投与群 201：111と差がなく（P＝0.58），血液生化学検査でも差はなかった。腫瘍径の中央値は両群ともに18 mmで差がなく，単発例はそれぞれの群で81.8%，85.6%とほぼ同

様であった。AFP の中央値はそれぞれ22 ng/mL，22 ng/mL，PIVKA-Ⅱ（Protein induced by vitamin K antagonist or absence）はそれぞれ3.6 AU/L，3.6 AU/L と同様であった。IFN 治療群の根治療法の方法は肝切除35例（45.4%）・RFA 42例（54.6%），非投与群では肝切除 153例（49.0%），RFA 159例（51.0%）と差はなかった。

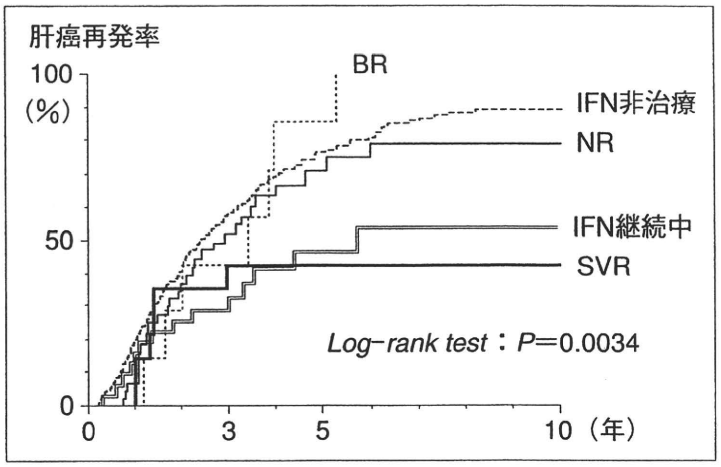

図3　インターフェロン治療効果別にみた肝癌再発率

IFN 治療の方法をみると，IFN＋リバビリンの併用療法が行われたのは9例（11.7%）で，他の68例（88.3%）はIFN 単独投与であった。IFN 投与期間は6ヵ月以内 10例，6ヵ月超1年以下 15例，1年超2年以下 13例，2年超5年以下 28例，5年超が11例であった。IFN 投与期間の中央値は2.1年（範囲 0.1～11.7年）で，2年以上継続使用例が39（50.6%）を占めた。

2．成　　績

1）IFN 投与の有無別にみた肝癌粗再発率（図2）

IFN 投与有無別に肝癌粗再発率を算出した。IFN 非投与群・投与群での肝癌再発率は，1年後がそれぞれ17.5%・9.1%，2年後が41.2%・33.3%，3年後が57.5%・41.1%，4年後が68.3%・58.8%，5年後が74.5%・63.0%で，IFN 投与群では明らかに再発率が低く，両群には有意差がみられた（log-rank test，P＝0.013）。

2）肝癌再発に寄与する独立要因

小型C型肝癌根治療法後の再発に寄与する独立要因を Cox 比例ハザードモデルで検討した。多変量解析の結果，再発に寄与する要因は，①IFN の投与あり（ハザード比 0.66，95%信頼限界 0.47-0.92，P＝0.015），②腫瘍多発（ハザード比 1.44，95%信頼限界 1.02-2.05，P＝0.040），③ICG 15分値 20%以上（ハザード比 1.42，95%信頼限界 1.11-1.83，P＝0.006），④肝癌治療法が RFA（ハザード比 1.33，95%信頼限界 1.03-1.71，P＝0.031）の4要因が挙げられた。IFNαの投与（投与群全体）により，非投与群に比し肝癌再発リスクが0.66に低下することが示された。

3）IFN 治療効果別にみた肝癌再発率（図3）

IFN の治療効果別にみた再発率曲線を IFN 未使用の群と比較して算出した。IFN の治療効果は，SVR 群（発癌前に IFN で HCV RNA 陰性化した10例も含む）14例，BR 群6例，NR 群 37例，投与中 30例に分け，IFN 未使用 302例と比較した。

SVR 群・BR 群・無効群・現在投与中・未投与群からの肝癌再発率はそれぞれ，1年で0%・0%・6.7%・12.5%・18.8%，2年で3.6%・28.6%・37.0%・25.3%・42.1%，3年で42.9%・42.9%・52.0%・32.6%・58.1%，4年で42.9%・85.7%・66.9%・41.3%・69.8%，5年での肝癌再発率はそれぞれ42.9%・85.7%・71.1%・46.7%・76.6%であった。全体での再発率は log-rank test で P＝0.0034で差がみられたが，SVR 群・IFN 継続

群での再発率が低い傾向であった。

3．考　案

IFNβによる肝癌再発抑制効果は，我々の報告[1]以外には発表されておらず，これまでの臨床成績に関しては，IFNαのデータがほとんどであり[7~12]，多くがretrospectiveな集計である。我々もretrospective研究ではあるが，IFNαによるC型肝癌根治療法後の再発抑制効果について検討した。大型肝癌・進行肝癌を使用した検討を行うと，腫瘍の不完全な治療・ablationによる再発やすでに起こっている肝内転移からの再発などがIFNの治療効果を曖昧にしてしまう可能性があり，この研究では腫瘍は3cm・3個以内の小型肝癌とし，内科治療はRFAのみに限定した。

肝癌粗再発率はIFN治療群で有意に低下し，多変量解析でも，IFN投与群では発癌リスクが0.66に低下することが示された。治療効果別には，SVR症例で肝癌再発率が低下するほか，長期のIFN治療継続を行っている群で再発率が低い傾向がみられた。

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Original Article

Development of HCC in patients receiving adefovir dipivoxil for lamivudine-resistant hepatitis B virus mutants

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Aim: To identify factors for the development of hepatocellular carcinoma (HCC) in the patients who receive adefovir add-on lamivudine for treatment of lamivudine-resistant hepatitis B virus (HBV) mutants.

Methods: A total of 247 patients who developed lamivudine-resistant HBV mutants, with an increase of HBV DNA ≥ 1 log copies/mL, received adefovir dipivoxil 10 mg add-on lamivudine 100 mg daily during a median of 115 weeks (range: 25–282 weeks). They were followed for the development of HCC by imaging modalities every 3–6 months.

Results: HCC developed in 18 of the 247 (7.3%) patients. Eight factors were in significant association with the development of HCC by the univariate analysis. They included age, cirrhosis, platelet counts, levels of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase and α-fetoprotein, as well as YMDD mutants at the start of adefovir dipivoxil. By the multivariate analysis, AST levels, YIDD mutants, cirrhosis and age were independent factors for the development of HCC. By the Kaplan-Meier analysis, AST levels ≥ 70 IU/L, YIDD mutants, cirrhosis and age ≥ 50 years increased the risk of HCC ($P = 0.018$, $P = 0.035$, $P = 0.002$ and $P = 0.014$, respectively). HCC developed more frequently in the patients with than without cirrhosis at the start of adefovir (10/59 [16.9%] vs. 8/188 [4.3%], $P = 0.002$).

Conclusion: HCC can develop in cirrhotic patients receiving adefovir add-on lamivudine. Hence, the patients with baseline AST ≥ 70 IU/L and YIDD mutants would need to be monitored closely for HCC.

Key words: adefovir dipivoxil, chronic hepatitis B, hepatitis B virus, hepatocellular carcinoma, lamivudine, rescue therapy

INTRODUCTION

WORLDWIDE, AN ESTIMATED 400 million people are infected with hepatitis B virus (HBV) persistently, and one million die of decompensated cirrhosis and/or hepatocellular carcinoma (HCC) annually.[1,2] Interferon (IFN) was introduced for treatment of chronic hepatitis B, and it has been replaced for pegylated-IFN.[3] Due to substantial side-effects and requirement for injection, however, IFN-based therapies are not favored.

In 1998, lamivudine was approved as the first nucleot(s)ide analogue for treatment of chronic hepatitis B,[4] and then adefovir in 2002.[5] Due to its lower costs and safety records, lamivdine has gained a wide popularity for treatment of chronic hepatitis B. However, drug-resistant mutants arise in parallel with the duration of lamivudine, in 12.5% after 1 year, in 43.8% after 3 years, and 62.5–70.2% after 5 years.[6,7] For preventing breakthrough hepatitis induced by lamivudine-resistant HBV mutants, additional adefovir dipivoxil 10 mg daily has been recommended;[8,9] it is more effective than switching to adefovir monotherapy and has fewer chances of developing drug-resistant mutants.[10,11]

Since 1995, 930 patients with chronic hepatitis have been treated with lamivudine in the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo.[12] HBV mutants with mutations in the thyrosine-methionine-aspartic acid-aspartic acid (YMDD) motif elicited in the 247 (26.5%) patients, and they started to receive additional adefovir since December, 2002.[13,14] However, HCC developed in 18 (7.3%) of them during the combination therapy for 25–282 weeks; HCC has

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not been reported in any of the patients who have received adefovir add-on lamivudine for 5 years.[15–17] Hence, factors for the development of HCC in the patients receiving adefovir add-on lamivudine were sought for in a retrospective study.

METHODS

Patients

OVER A PERIOD of 13 years, from September 1995 to September 2007, 930 patients with chronic hepatitis B received long-term lamivudine treatment at the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. Drug-resistant YMDD mutants developed in 247 (26.5%) of them, accompanied by an increase in HBV DNA ≥ 1 log copies/mL, and they received adefovir 10 mg in addition to lamivudine 100 mg daily during the median of 115 weeks (range: 25–282 weeks). They have been followed for liver function and virological markers of HBV infection monthly, as well as blood counts and tumor makers including alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II). Cirrhosis was diagnosed by laparoscopy or liver biopsy, and in the patients who had not received them, by clinical data, imaging modalities and portal hypertension. HCC was diagnosed by hypervascularity on angiography and/or histological examination, characteristic features of computed tomography, magnetic resonance imaging and ultrasonography. An informed consent was obtained from each patient in this study, and the protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a *priori* approval by the institution's human research committee.

Markers of HBV infection

Hepatitis B e antigen (HBeAg) was determined by enzyme-linked immunosorbent assay (ELISA) with commercial kits (HBeAg EIA, Institute of Immunology, Tokyo). HBV DNA was quantitated by the Amplicor monitor assay (Roche Diagnostics, Tokyo) with a dynamic range over 2.6–7.6 log copies/mL. Genotypes of HBV were determined serologically by the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the seven major genotypes (A–G),[18,19] with use of commercial kits (HBV Genotype EIA, Institute of Immunology).

Detection of YMDD mutants

YMDD mutants were determined by polymerase chain reaction (PCR)-based enzyme-linked mini-sequence assay (PCR-ELIMA) with commercial kits (Genome Science Laboratories, Tokyo).

Statistical analyses

Categorial variables were compared between groups by the χ^2 test, and non-categorical variables by the Mann–Whitney *U*-test. A *P*-value < 0.05 was considered significant. Factors associated with HCC by univariate analysis were evaluated by the multivariate analysis by the stepwise Cox proportional hazard model. Development of HCC with time was analyzed by the Kaplan–Meier method, and differences were evaluated by the log-rank test. Data were analyzed by the SPSS software, version 11.0 (Chicago, IL).

RESULTS

Baseline characteristics of the patients who did and who did not develop hepatocellular carcinoma during adefovir add-on lamivudine treatment

TABLE 1 COMPARES characteristics at the start of adefovir between the 18 patients who developed HCC and the 229 who did not. Eight factors were associated with the development of HCC by the univariate analysis. They included age, cirrhosis, platelet counts, bilirubin, AST, alanine aminotransferase (ALT) and α-fetoprotein (AFP) levels, as well as YMDD mutants. HCC developed more frequently in the patients with than without cirrhosis at the start of adefovir (10/59 [16.9%] vs. 8/188 [4.3%], $P = 0.002$). There were 61 (26.6%) patients who had cirrhosis at the start of adefovir. Of them, one of the 18 (2.2%) with HCC and 18 of the 229 (2.2%) without HCC presented with decompensation; no patients developed decompensation after the start of adefovir.

Rates of HBV DNA disappearance from serum (< 2.6 log copies/mL) were: 55% (113/207) at 1 year, 71% (119/168) at 2 years, 77% (78/101) at 3 years and 85% (35/41) at 4 years. Rates of AST nomarlization (< 38 IU/L) were: 87% (179/207) at 1 year, 90% (151/168) at 2 years, 92% (93/101) at 3 years and 95% (39/41) at 4 years; and those of ALT normalization (< 50 IU/L) were: 88% (183/207) at 1 year, 91% (153/168) at 2 years, 93% (94/101) at 3 years and 98% (40/41) at 4 years. There were no differences in the rate of HBV DNA disappearance from serum between the patients with and without HCC: 57% (8/14) vs. 54% (105/193) at 1 year ($P = 1.0$); 86% (12/14) vs. 70% (107/154) at 2 years ($P = 0.229$); and 89% (8/9) vs.

Table 1 Characteristics of patients who did and did not develop hepatocellular carcinoma (HCC) at the start of adefovir†

	HCC developed ($n = 18$)	HCC did not develop ($n = 229$)	Differences *P*-value
Duration of lamivudine before the start of adefovir	128 (31–346)	144 (13–617)	0.321
Age (years)	52 (35–75)	45 (26–75)	0.008
Men	15 (83%)	183 (80%)	1.000
Cirrhosis	10 (56%)	51 (22%)	0.004
Platelets ($\times 10^3/mm^3$)	12.0 (4.6–19.7)	16.3 (3.1–31.9)	0.001
Albumin (g/dL)	3.6 (2.3–4.7)	3.9 (2.8–4.7)	0.073
Bilirubin (mg/dL)	0.8 (0.5–15.5)	0.7 (0.2–6.0)	0.046
Creatinine (mg/dL)	0.8 (0.5–1.0)	0.8 (0.4–1.6)	0.950
AST (IU/L)	119 (55–248)	66 (14–1413)	0.003
ALT (IU/L)	151 (61–576)	104 (13–1563)	0.035
AFP (ng/dL)	8 (2–130)	4 (1–282)	0.026
HBV genotypes			0.228
C	18 (100%)	189 (87%)	
Others	0	27 (13%)	
HBeAg	8 (44%)	132 (58%)	0.323
HBV DNA (log copies/mL)	7.1 (4.4–>7.6)	7.1 (<2.6–>7.6)	0.623
YMDD mutants			0.041
YIDD	13 (72%)	109 (45%)	
YVDD	5 (28%)	62 (25%)	
YI/VDD	0	56 (23%)	

†Values are the median with the range in parentheses or *n* with percent in parentheses.
AFP, alpha-fetoprotein; ALT, alaine aminotransferase; AST, aspartate aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

92% (85/92) at 3 years ($P = 0.555$). Rates of normalized AST levels in the patients with and without HCC were: 50% (7/14) vs. 90% (173/193) at 1 year ($P < 0.001$); 79% (11/14) vs. 91% (140/154) at 2 year ($P = 0.166$); and 67% (6/9) vs. 95% (87/92) at 3 year ($P = 0.037$). Rates of ALT normalization in the patients with and without HCC were: 71% (10/14) vs. 90% (174/193) at 1 year ($P = 0.037$); 79% (11/14) vs. 90% (139/154) at 2 year ($P = 0.189$); and 56% (5/9) vs. 92% (85/92) at 3 year ($P = 0.015$). Thus, normalization of AST and ALT was less frequent in the patients with than without HCC.

Characteristics of the 18 patients who developed HCC are compared between the baseline and at the development of HCC (Table 2). At the start of adefovir, 10 (56%) of them had developed cirrhosis and 16 (89%) had AST levels ≥ 70 IU/L. HBV DNA was not detectable in 10 (56%) of them at the development of HCC. Of the eight patients with detectable HBV DNA levels (≥ 2.6 log copies/mL), five (63%) developed HCC within 1 year after the start of adefovir. AST was elevated (> 38 IU/L) in eight patients, including four (50%) without detectable HBV DNA levels.

Factors independently associated with the development of hepatocellular carcinoma

Eight factors associated with the development of HCC by the univariate analysis, including age, cirrhosis, platelet counts, bilirubin, AST, ALT and AFP levels, as well as YMDD mutants (Table 1), were evaluated by the multivariate analysis. AST ≥ 70 IU/L, YIDD mutants, age ≥ 50 years and cirrhosis at the baseline were independent risk factors for the development of HCC (Table 3). There were no differences in the distribution of YIDD, YVDD and the mixture thereof among the patients with distinct AST, ALT or HBV DNA levels or between those with and without cirrhosis at the start of adefovir. HBV mutants with mutations resistant to adefovir (rtA181T/S, rtN236T) occurred in two of the 247 (0.8%) patients; none of them developed HCC.

The median time between the elevation of HBV DNA > 5.0 log copies/mL and the administration of adefovir was 124 (range: 0–815) days for the 13 patients who developed HCC and 147 (0–3268) days for the 166 patients who did not ($P = 0.605$). The median time between the elevation of ALT > 43 IU/L and the start of

Table 2 Characteristics of the 18 patients at commencement of adefovir (ADV) and development of hepatocellular carcinoma (HCC)

Patient no.	Age (years)	Sex	At the commencement of ADV						Period of ADV (years)	At the development of HCC		
			Liver disease	AST (IU/L)	ALT (IU/L)	HBeAg	HBV DNA (log copies/mL)	YMDD mutant		AST (IU/L)	ALT (IU/L)	HBV DNA (log copies/mL)
1	50	M	CH	248	576	−	6.9	I	4.5	26	27	< 2.6
2	35	M	LC	217	164	+	7.5	I	1.6	54	34	< 2.6
3	50	M	LC	192	272	+	> 7.6	I	1.2	68	89	< 2.6
4	61	M	CH	192	332	−	6.9	I	2.8	22	23	< 2.6
5	65	M	CH	174	219	−	5.2	V	0.1	30	43	< 2.6
6	58	M	CH	160	216	−	6.5	V	2.2	41	32	< 2.6
7	53	M	LC	127	97	+	> 7.6	I	0.5	55	41	3.2
8	75	M	LC	119	209	+	> 7.6	V	1.1	121	125	2.6
9	58	F	CH	118	214	+	4.4	I	3.3	21	13	< 2.6
10	48	M	CH	116	99	+	> 7.6	I	3.3	32	36	< 2.6
11	51	F	LC	111	130	−	5.3	I	0.9	88	95	< 2.6
12	47	M	CH	85	138	+	> 7.6	I	1.3	28	29	3.1
13	61	M	LC	81	65	−	5.6	I	0.2	32	27	2.9
14	59	F	LC	80	132	−	> 7.6	V	0.1	32	41	3.2
15	40	M	LC	75	124	−	6.3	I	3.8	21	24	< 2.6
16	48	M	CH	71	61	−	6.6	I	0.6	48	26	3.7
17	55	M	LC	55	76	+	7.3	I	0.2	50	64	5.4
18	43	M	LC	27	21	−	5.4	V	1.6	30	23	3.7

ALT, alaine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; I, YIDD mutant; LC, cirrhosis; V, YVDD mutant.

Table 3 Independent risk factors influencing the development of hepatocellular carcinoma

Factors	Category	Hazard ratio (95% CI†)	*P*-value
AST (IU/l)	1: < 70	1	0.016
	2: ≥ 70	6.21 (1.40–27.5)	
YMDD mutants	1: YVDD or YV/IDD	1	0.012
	2: YIDD	3.97 (1.36–11.6)	
Age (years)	1: < 50	1	0.023
	2: ≥ 50	3.24 (1.17–8.95)	
Cirrhosis	1: Absent	1	0.030
	2: Present	1.42 (1.04–1.96)	

†Confidence interval.

adefovir was 59 (0–896) days for the patients who developed HCC and 54 (0–3240) days for those who did not ($P = 0.330$). Hence, exacerbation of hepatitis was not a risk factor for the development of HCC.

Age-specific risk factors for the development of HCC were evaluated by the multivariate analysis. In the patients < 50 years, platelet counts < $13 \times 10^3/mm^3$ was the only significant risk factor for HCC (hazard ratio 6.88 [95% confidence interval; 1.26–37.6]), while AST levels ≥ 70 IU/L was that in those ≥ 50 years (hazard ratio: 9.50 [95% confidence interval 1.20–74.9]).

Factors increasing the cumulative incidence of hepatocellular carcinoma

AST levels ≥ 70 IU/L at the start of adefovir increased the development of HCC during follow-ups ranging to 5 years (Fig. 1). HCC developed more frequently in the patients with YIDD mutants than in those with YVDD or the mixture of YVDD and YIDD mutants (Fig. 2). The cumulative incidence of HCC in the patients with YIDD mutants alone was: 4% at 1 year, 10% at 3 years and 43% at 5 years. In contrast, HCC never developed in the patients with the mixture of YIDD and YVDD mutants through 5 years of follow-up. HCC developed more frequently in the patients with cirrhosis and those aged ≥ 50 years (Figs 3,4, respectively).

DISCUSSION

HCC DEVELOPED IN 18 of the 247 (7.3%) patients who had received adefovir add-on lamivudine during a long-term ranging to 5 years. There were some differences in the characteristics at the start of adefovir dipivoxil between the patients who did and who did not develop HCC. The patients who developed HCC were older, more frequently had signs of early cirrhosis with less platelet counts, as well as higher levels of AST, ALT and AFP, than those who did not develop HCC. By multivariate analysis, AST ≥ 70 IU/L, YIDD mutants in

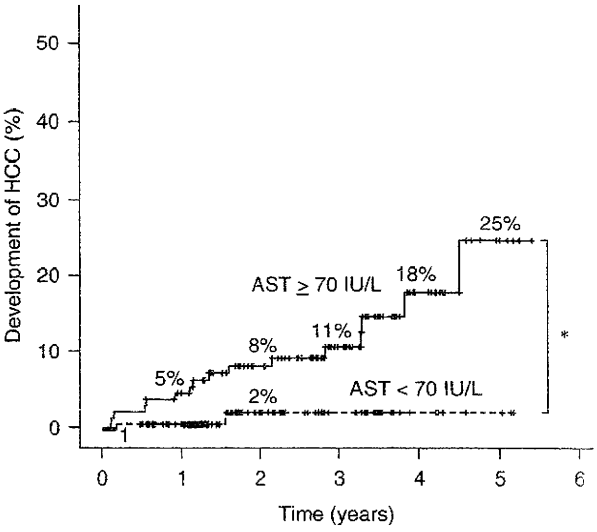

Figure 1 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients with different baseline aspartate aminotransferase (AST) levels. *$P = 0.009$.

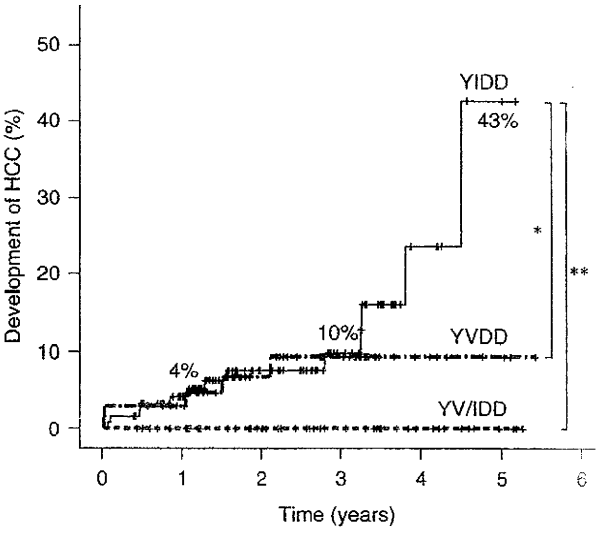

Figure 2 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients with distinct YMDD mutants. *$P = 0.035$; **$P = 0.003$.

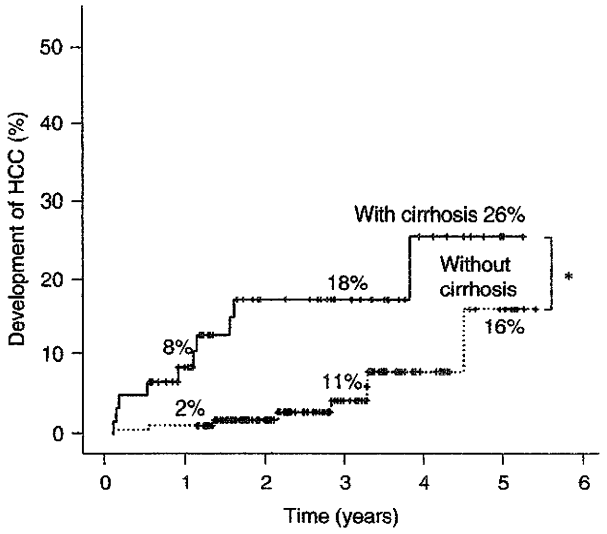

Figure 3 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients with and without cirrhosis at the baseline. $^*P = 0.002$.

comparison with YVDD or the mixture of YVDD and YIDD mutants, age ≥ 50 years and cirrhosis were independent risk factors for the development of HCC. By the Kaplan-Meier life-table analysis, the cumulative incidence of HCC during 5 years in the patients receiving

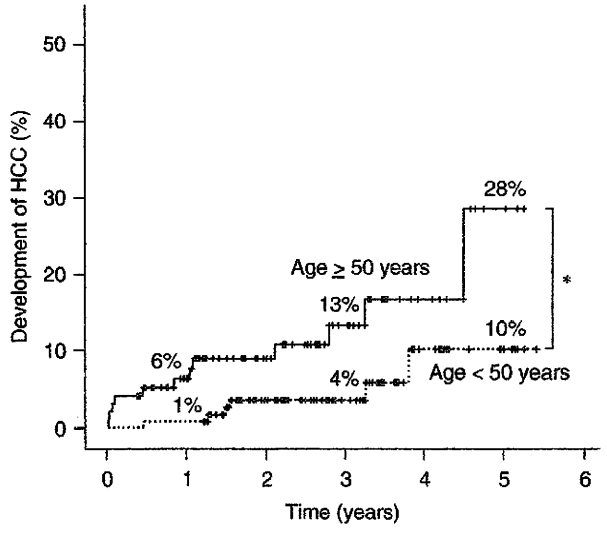

Figure 4 Kaplan–Meier life-table for the cumulative incidence of hepatocellular carcinoma (HCC) during adefovir add-on lamivudine in the patients aged ≥ 50 years and < 50 yeas at the baseline. $^*P = 0.014$.

adefovir add-on lamivudine was significantly higher in those with AST ≥ 70 IU/L, YIDD mutants, cirrhosis and aged ≥ 50 years at the start of adefovir.

A marked difference in the development of HCC between the present study (7.3% [18/247]) and two studies reported from Europe and the US (0/70 and 0/65, respectively)[16,17] would be accounted for, at least in part, by the age of patients who developed HCC in this study that was older than in those in previous reports (the median of 52 years vs. means of 36 and 47 years, respectively). This view would be supported by the age of patients with long-term adefovir add-on lamivudine that was higher in those with than without the development of HCC (52 vs. 45 years [median], $P = 0.008$). HBV infection in Asia is acquired by the perinatal infection, while that in Western countries is gained after the adolescence ~20 years after birth. Hence, the duration of HBV infection would have been > 20 years longer in Japanese than Western patients. In addition, genotypes of HBV may give an additional account on the difference in development of HCC between them. All the 18 patients who developed HCC in this study were infected with genotype C; it is associated with HCC more closely than the other genotypes.[20–23] By contrast, by far the most patients from Western countries would have been infected with genotypes A and D.[24,25]

HCC developed more frequently in patients with than without cirrhosis at the start of adefovir (10/61 [16.4%] vs. 8/186 [4.3%], $P = 0.002$). Hence, cirrhosis increased the risk of HCC in patients receiving adefovir add-on lamivudine. This view is supported by the development of HCC in 11 of the 94 (11.7%) patients with cirrhosis who received adefovir add-on lamivudine from Italy.[10] Although HCC did not develop in any of the 39 Italian patients with chronic hepatitis, it did in eight of the 186 (4.3%) Japanese patients in the present study. There were, however, marked differences in the median baseline ALT levels between Italian and Japanese patients (58 vs. 108 IU/L); the grade of liver inflammation would have been higher in the Japanese patients. In actuality, all the eight patients with chronic hepatitis who developed HCC had high AST and ALT levels at the start of adefovir (Table 2).

In the natural history of persistent HBV infection, HCC develops more frequently in the patients with persistently high ALT levels than in those with normal levels. Hence, necroinflammation in the liver would contribute to carcinogenesis.[26,27] Although adefovir add-on lamivudine may prevent virological breakthroughs, it would not be able to suppress the pre-

neoplastic state induced by exacerbation of hepatitis. It would be necessary therefore to identify the patients with chronic hepatitis at an increased risk for HCC during adefovir add-on lamivudine, such as those with cirrhosis or aged ≥ 50 years, and take special care of them toward early detection of HCC and immediate therapeutic intervention. They need to be monitored frequently for any increase in HBV DNA and aminotransferase levels that herald breakthrough hepatitis during lamivudine therapy.

In the present study, HCC developed more frequently in the patients with YIDD mutants than in those with YVDD or the mixture of YVDD and YIDD; there have been no studies correlating YMDD mutants and the development of HCC. No patients with the mixture of YVDD and YIDD mutants developed HCC, despite the predominance of YIDD mutants in the patients with HCC. This might have been due to the assay used for YMDD mutants by the commercial kit; it can miss YVDD mutants in samples in which YIDD mutants account for the great majority. By the assay method specific for either mutant, YIDD was detected either alone or accompanied by small amount of YVDD in the patients who have received adefovir add-on lamivudine treatment.[28] Sensitive and specific quantification of YIDD and YVDD mutants are necessary for further evaluating a role for YIDD mutants in hepatocarcinogenesis, as well as for identifying factors promoting the generation of both YIDD mutants and HCC.

Some points of clinical importance have emerged in the present study. First, patients who receive a long-term adefovir add-on lamivudine and have developed YMDD mutants need to be screened for HCC on the regular basis. This is required especially for the patients who have signs of cirrhosis and/or high AST levels, or aged ≥ 50 years. In these high-risk patients, adefovir has to be started promptly when HBV DNA levels increase, even before transaminase levels elevate in them. Secondly, it would be a matter of concern if adefovir is involved in the development of HCC. Should it be the case, tenofovir or newer potent antivirals, either as a monotherapy or add-on lamivudine, would deserve considerations. Thirdly, it needs to be evaluated if YIDD mutants have any significance in the development of HCC. Although nucleot(s)ide analogues may suppress hepatic inflammation and are expected to improve the prognosis of patients with chronic hepatitis B, they need to be monitored closely for HCC. The development of HCC has to be identified, as early as possible, for timely treatment toward longevity with minimal morbidity and improvement of the quality of life.

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HEPATOLOGY

Efficacy of switching to entecavir monotherapy in Japanese lamivudine-pretreated patients

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Abstract

Background and Aims: To assess the efficacy of switching Japanese chronic hepatitis B patients from lamivudine monotherapy to entecavir 0.5 mg/day.

Methods: A retrospective analysis was conducted on 134 patients switched to entecavir between September 2006 and February 2008 for 6 months or more. Patients were divided into three groups based on viral load at entecavir switching point (baseline < 2.6, 2.6–5.0 and > 5.0 $\log_{10}$ copies/mL).

Results: At baseline, detection of lamivudine-resistant virus was highest in patients with higher hepatitis B virus (HBV) DNA (76% *vs* 23% in ≥ 2.6 and < 2.6 $\log_{10}$ copies/mL, respectively), and in patients with longest previous exposure to lamivudine (52%, 28% and 24% for > 3 years, 1–3 years and < 1 year, respectively). Two years after entecavir switching, HBV DNA suppression to less than 2.6 $\log_{10}$ copies/mL was achieved in 100% (32/32), 92% (12/13) and 44% (4/9) of patients in the less than 2.6, 2.6–5.0 and more than 5.0 $\log_{10}$ copies/mL baseline groups, respectively. Alanine aminotransferase (ALT) normalization occurred in 76–96% and 90–100% of patients following 1 and 2 years of entecavir treatment, respectively. One patient (2.6–5.0 $\log_{10}$ copies/mL) with lamivudine-resistant mutants at baseline developed entecavir resistance at week 48 during follow up.

Conclusion: Switching to entecavir 0.5 mg/day achieves or maintains undetectable HBV DNA levels and ALT normalization over 2 years, especially in patients with viral load less than 5.0 $\log_{10}$ copies/mL.

Introduction

Hepatitis B virus (HBV) infection is a serious public health threat affecting 350–400 million people worldwide, the majority of whom live in the Asia–Pacific region.[1,2] Chronically-infected people are at risk of developing cirrhosis, liver failure and hepatocellular carcinoma. Studies have suggested that high serum HBV DNA is a key risk predictor of chronic hepatitis B (CHB) complications.[3,4] Therefore, the main purpose of CHB therapies is to permanently suppress viral replication and sustain viral suppression to prevent long-term liver damage.[2,5,6]

Lamivudine was the first nucleoside analog to be widely prescribed for CHB patients, mainly due to its antiviral efficacy and safety profile.[2] However, lamivudine's long-term efficacy is diminished by the emergence of drug-resistant substitutions, generally in the tyrosine–methionine–aspartate–aspartate (YMDD) motif of the reverse transcriptase (rt) polymerase gene.[7–9] Detection of lamivudine-resistant HBV substitutions occurs in 15–30% and 70% of patients after 1 and 5 years of treatment, respectively.[8] Continuing lamivudine monotherapy in the presence of lamivudine resistance is not recommended because it is no longer effective in suppressing viral replication.[2] Furthermore, the initial improvement in histology and clinical benefits may be reversed or decreased due to the emergence of lamivudine-resistant substitutions.

Antiviral efficacy of entecavir (0.5 mg/day) as first-line therapy was superior to lamivudine in treatment-naïve patients on all virological, biochemical and histological end-points after 48 weeks of treatment,[10–14] with very low rates of emergence of viral resistance (1.2% after 5 years of entecavir treatment).[15,16] Entecavir has a high genetic barrier to resistance,[17–19] requiring multiple substitutions (including YMDD mutations) to express viral resistance.[16–21] In agreement with this, entecavir-resistant mutants emerge more frequently in lamivudine-refractory patients.[22,23] In a study of hepatitis B e antigen (HBeAg)-positive lamivudine-refractory patients with high HBV DNA levels at baseline (mean > 9 $\log_{10}$ copies/mL), switching to entecavir 1 mg/day achieved HBV DNA suppression to undetectable levels (< 300 copies/mL; 40%, 96 weeks) and alanine aminotransferase (ALT) normalization (81%, 96 weeks) at higher proportions than continued lamivudine

monotherapy,[22] although response to therapy was less pronounced than in treatment-naïve patients with comparable baseline levels of HBV DNA.[10,13,14] The probability of achieving HBV DNA suppression to undetectable levels at 96 weeks with entecavir was 73% in patients whose baseline HBV DNA was less than 7 $\log_{10}$ copies/mL ($n = 11$), and none of these patients developed entecavir resistance.[22]

In a randomized controlled trial of lamivudine-refractory Japanese patients with mean HBV DNA at baseline of 7.6–7.7 $\log_{10}$ copies/mL, switching to entecavir (0.5 or 1 mg/day) for 48 weeks achieved HBV DNA suppression to below detectable levels in 33% of patients in the entecavir dose groups, and ALT normalization in 78–86%.[24] Switching to entecavir in patients with evidence of lamivudine-resistant substitutions and low viral load at switching point has not been prospectively investigated in Japanese patients. There are limited data concerning the efficacy of entecavir in lamivudine-pretreated patients who have not developed lamivudine resistance.

The objective of this study was to assess the efficacy of switching to entecavir 0.5 mg/day in Japanese lamivudine-pretreated patients whose HBV DNA levels at switching point (baseline) ranged from less than 2.6 to 7.6 $\log_{10}$ copies/mL, with or without lamivudine-resistant substitutions.

Methods

Design and setting

A retrospective analysis of a CHB patient population ($n = 134$) at Toranomon Hospital (Tokyo, Japan) was performed to identify patients switched from lamivudine 100 mg/day monotherapy to entecavir 0.5 mg/day between September 2006 and February 2008, and who had received entecavir for at least 6 months. Among all patients selected, only one had a history of adefovir add-on therapy prior to switching to entecavir (case report). Conserved serum from all patients was analyzed to determine baseline characteristics and study end-points.

Study end-points

Clinical efficacy of entecavir was assessed as the proportion of patients achieving HBV DNA suppression to undetectable levels (< 400 copies/mL or < 2.6 $\log_{10}$ copies/mL), and patients achieving ALT normalization (normal ALT levels: men 8–42 IU/L, women 6–27 IU/L). HBV DNA was measured using the polymerase chain reaction (PCR)-based Amplicor HBV Monitor assay (Roche Diagnostics, Indianapolis, IN, USA; lower limit of detection of < 2.6 $\log_{10}$ copies/mL).[25] HBeAg loss in patients who were HBeAg-positive at baseline was also analyzed. Measurements were made from conserved samples taken at baseline, and after 6 months, 1 and 2 years from entecavir treatment initiation.

Assessment of viral resistance

Conserved serum was used to detect the presence of viral lamivudine-resistant rtM204V/I substitutions in all patients at baseline, and following the entecavir switch in patients treated with entecavir for at least 6 months. Lamivudine-resistant virus (rtM204V/I or YMDD motif substitutions) was analyzed using a combination of the quantitative enzyme-linked immunosorbent assay standardized using a purified *Taenia solium* cysticerci fraction (PCR enzyme-linked immunosorbent assay) and the enriched PCR enzyme linked minisequence assay.[26] Direct sequencing of HBV DNA polymerase reverse transcriptase site was also performed.[27] Detection of entecavir-resistant virus was conducted using direct sequencing of HBV DNA polymerase reverse transcriptase site.[27]

Data analyses

Statistical comparisons between treatment groups were assessed using χ^2-test and Kruskal–Wallis test where appropriate. Calculations were performed using StatView software (ver. 4.5J; Abacus Concepts, Berkeley, CA, USA). A two-tailed P-value less than 0.05 was considered statistically significant.

To identify predictive factors of HBV DNA negativity (suppression to below detectable levels) after 6 months of the entecavir switch, univariate and multivariate logistic regression analyses were carried out. Potential predictive factors at baseline included: sex; age; levels of aspartate aminotransferase (AST), ALT, albumin, γ-glutamyl transpeptidase, total bilirubin and α-fetoprotein; platelet count; viral load; liver disease stage (cirrhosis or other); family history; HBV genotype; lamivudine treatment duration prior to entecavir switch; HBeAg status; and lamivudine resistance. Each variable was transformed into categorical data consisting of two simple ordinal numbers. All factors that were at least marginally associated with HBV DNA negativity ($P < 0.10$) were used in a multiple logistic regression analysis. To assess relative risk confidence, odds ratio (OR) and 95% confidence interval (CI) were calculated. All analyses were performed using SPSS II software ver. 11.0 (SPSS, Chicago, IL, USA).

Results

Patient characteristics before switching to entecavir

Lamivudine-pretreated patients switched to entecavir 0.5 mg/day ($n = 134$) were divided into three groups based on their HBV DNA level at the switching point: HBV DNA of less than 2.6 $\log_{10}$ copies/mL ($n = 92$), 2.6–5.0 $\log_{10}$ copies/mL ($n = 25$) and more than 5.0 $\log_{10}$ copies/mL ($n = 17$) (Table 1). Patients with HBV DNA levels of more than 5.0 $\log_{10}$ copies/mL had the highest AST/ALT levels and highest proportion of HBeAg-positive cases ($P < 0.05$). These patients had been treated with lamivudine for the shortest time period compared to patients from the two other groups ($P < 0.05$; Table 1).

Viral resistance to lamivudine at baseline

At baseline, lamivudine-resistant rtM204V/I mutant virus was detected in 23% of patients with HBV DNA of less than 2.6 $\log_{10}$ copies/mL, compared to 76% in each of the HBV DNA 2.6–5.0 $\log_{10}$ copies/mL and more than 5.0 $\log_{10}$ copies/mL groups (Table 2). In all treatment groups, a higher occurrence of resistant virus was observed with longer exposure to lamivudine, independent of viral DNA levels.

Table 1 Patient characteristics at point of switching to entecavir (baseline) and entecavir treatment duration

	All patients	Serum HBV DNA levels by baseline treatment group, log_{10} copies/mL			
		< 2.6	2.6–5.0	> 5.0	*P**
Patients, *n*	134	92	25	17	
Sex, *n* male/female	94/40	67/25	19/6	8/9	0.08
Age, years†	53 (23–83)	53 (27–83)	50 (32–77)	37 (23–77)	0.036
Bilirubin, mg/dL†	0.6 (0.2–3.4)	0.6 (0.2–3.4)	0.6 (0.3–1.8)	0.7 (0.3–1.2)	0.53
AST, IU/L†	24 (13–451)	23 (13–53)	23 (14–50)	37 (14–451)	0.0083
ALT, IU/L†	21 (8–1382)	21 (8–56)	20 (10–111)	46 (9–1382)	0.0002
Albumin, g/dL†	3.9 (2.7–4.8)	3.9 (2.7–4.4)	4.0 (3.3–4.8)	3.9 (3.6–4.6)	0.94
Histology, *n* CH/LC	89/45	56/36	19/6	14/3	0.11
HBeAg, *n* ±	30/104	11/81	5/20	14/3	< 0.0001
HBV DNA, log_{10} copies/mL†	< 2.6 (< 2.6–7.6)	< 2.6	3.9 (2.7–5.0)	6.5 (5.1–7.6)	–
Genotype, *n* A/B/C/unknown	3/9/115/7	2/6/78/6	1/2/22/0	0/1/15/1	0.87
Treatment duration, months†					
Lamivudine	36 (0.5–103)	36 (3–103)	70 (2–89)	17 (0.5–89)	0.009
Entecavir‡	21 (6–33)	20 (6–33)	24 (6–32)	27 (6–33)	0.034

*Comparison of the three patient subgroups using the Kruskal–Wallis test; $P < 0.05$ was considered statistically significant.

†Data are median (range).

‡Entecavir treatment duration is from point of switching.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; LC, liver cirrhosis.

Table 2 rtM204V/I mutant occurrence at baseline of switching to entecavir

	Duration of previous lamivudine treatment, years			All patients
	< 1	1–3	≥ 3	
Baseline treatment group				
< 2.6 log_{10} copies/mL	1/10 (10%)	4/35 (11%)	16/47 (34%)	23%
2.6–5.0 log_{10} copies/mL	1/5 (20%)	3/4 (75%)	15/16 (94%)	76%
> 5.0 log_{10} copies/mL	3/6 (50%)	6/7 (86%)	4/4 (100%)	76%
All patients	24%	28%	52%	–

Clinical efficacy of entecavir 0.5 mg/day

Switching to entecavir 0.5 mg/day for 1 year resulted in HBV DNA suppression to undetectable levels in the majority of patients with HBV DNA below 5.0 log_{10} copies/mL (100% and 96% for HBV DNA < 2.6 and 2.6–5.0 log_{10} copies/mL, respectively) (Table 3). This proportion was slightly decreased when previous lamivudine treatment duration exceeded 3 years in the 2.6–5.0 log_{10} copies/mL group. In the HBV DNA more than 5.0 log_{10} copies/mL group, approximately half (41%) of the patients achieved viral suppression after 1 year (Table 3); entecavir's efficacy seemed to decrease with prolonged previous exposure to lamivudine, with only 25% of patients having more than 3-year lamivudine treatment achieving undetectable viral load. Similarly, after 2 years, HBV DNA suppression was achieved by 100% and 92% of patients in the HBV DNA less than 2.6 and 2.6–5.0 groups, respectively, and by 44% of patients in the HBV DNA more than 5.0 log_{10} copies/mL group (Table 3).

Among those who failed to suppress viral load, only one case of virological breakthrough was found (2.6–5.0 log_{10} copies/mL group; described under case report). This patient had been previously exposed to lamivudine for more than 3 years.

Alanine aminotransferase levels were normalized in 76–96% and 90–100% of patients following 1 and 2 years of entecavir treatment, respectively (Table 3). HBeAg loss was observed in 27% (3/11), 20% (1/5) and 29% (4/14) of patients with HBV DNA of less than 2.6, 2.6–5.0 and more than 5.0 log_{10} copies/mL, respectively, in the first year.

Lamivudine-resistant substitutions in patients switched to entecavir

Of the 130 patients who received entecavir treatment for at least 1 year, 11 cases failed to suppress HBV DNA to below less than 2.6 log_{10} copies/mL and remained HBV DNA-positive in the first year (1 and 10 in the HBV DNA 2.6–5.0 and > 5.0 log_{10} copies/mL groups, respectively; Table 3). Serum HBV DNA analysis confirmed the presence of rtM204V/I substitutions in 10 of these patients, of which six were rtM204I and three were rtM204V substitutions (Table 4); the remaining patient (2.6–5.0 log_{10} copies/mL group; previous lamivudine exposure 5 years) carried a mixed type substitution, rtM204I plus rtM204V. The only HBV DNA-positive patient who did not

Table 3 Clinical efficacy of entecavir 0.5 mg/day in lamivudine-pretreated patients

End-point by baseline treatment group	Duration of entecavir treatment		
	6 months	1 year	2 years
HBV DNA suppression to undetectable levels, *n*/N (%)			
< 2.6 $\log_{10}$ copies/mL	90/92 (98%)	89/89 (100%)	32/32 (100%)
Previous lamivudine < 1 year	10/10 (100)	9/9 (100)	5/5 (100)
Previous lamivudine 1–3 years	35/35 (100)	35/35 (100)	14/14 (100)
Previous lamivudine > 3 years	45/47 (96)	45/45 (100)	13/13 (100)
2.6–5.0 $\log_{10}$ copies/mL	24/25 (96%)	23/24 (96%)	12/13 (92%)
Previous lamivudine < 1 year	5/5 (100)	5/5 (100)	3/3 (100)
Previous lamivudine 1–3 years	4/4 (100)	4/4 (100)	2/2 (100)
Previous lamivudine > 3 years	15/16 (94)	14/15 (93)	7/8 (88)
> 5.0 $\log_{10}$ copies/mL	5/17 (29%)	7/17 (41%)	4/9 (44%)
Previous lamivudine < 1 year	2/6 (33)	3/6 (50)	2/4 (50)
Previous lamivudine 1–3 years	2/7 (29)	3/7 (43)	2/4 (50)
Previous lamivudine > 3 years	1/4 (25)	1/4 (25)	0/1 (0)
ALT normalization, *n*/*n* (%)			
< 2.6 $\log_{10}$ copies/mL	88/92 (96%)	83/89 (93%)	32/32 (100%)
2.6–5.0 $\log_{10}$ copies/mL	24/25 (96%)	23/24 (96%)	12/13 (92%)
> 5.0 $\log_{10}$ copies/mL	14/17 (82%)	13/17 (76%)	9/10 (90%)

ALT, alanine aminotransferase; HBV, hepatitis B virus.

Table 4 HBV DNA positive rates in patients switched to entecavir 0.5 mg/day for at least 1 year

	HBeAg status	YMDD motif substitution	HBV DNA positive rate, *n*/N (%)	Duration of previous lamivudine treatment, years per patient
Baseline treatment group				
< 2.6 $\log_{10}$ copies/mL	Positive	Wild (or none)	0/10 (0%)	n/a
		YIDD	0/1 (0%)	n/a
	Negative	Wild (or none)	0/58 (0%)	n/a
		YIDD	0/15 (0%)	n/a
		YVDD	0/4 (0%)	n/a
		YIDD + YVDD	0/1 (0%)	n/a
2.6–5.0 $\log_{10}$ copies/mL	Positive	Wild (or none)	0/4 (0%)	
		YIDD + YVDD	1/1 (100%)[†]	5.0
	Negative	Wild (or none)	0/2 (0%)	n/a
		YIDD	0/10 (0%)	n/a
		YVDD	0/6 (0%)	n/a
		YIDD + YVDD	0/1 (0%)	n/a
> 5.0 $\log_{10}$ copies/mL	Positive	Wild (or none)	1/4 (25%)	0.2
		YIDD	6/9 (67%)	0.5; 1.3; 1.5; 2.7; 3.9; 7.4
		YVDD	1/1 (100%)	0.7
	Negative	YIDD	0/1 (0%)	n/a
		YVDD	2/2 (100%)	1.8; 4.5
All patients			11/130 (8%)	

YMDD motif substitutions: wild, rt204M; YIDD, rt204I; YVDD, rt204V; YIDD + YVDD, rt204I + rt204V.
[†]Patient with lamivudine-resistant HBV who developed entecavir resistance.
HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; n/a, not available.

carry any detectable lamivudine-resistant substitution had the shortest previous lamivudine exposure (< 6 months; Table 4).

Of the 10 patients carrying rtM204V/I substitutions, eight were HBeAg-positive; the other two patients were HBeAg-negative and carried a lamivudine-resistant rtM204V type substitution.

Emergence of entecavir-resistant mutant: case report

One patient (2.6–5.0 $\log_{10}$ copies/mL group) carrying a mixed substitution YIDD + YVDD (rtM204I + rtM204V) developed entecavir resistance with a recognized rtS202G substitution