Ⅶ. 肝移植

2．肝移植後の抗ウイルス治療

はじめに

肝硬変患者の多くがB型肝炎ウイルス(HBV)，またはC型肝炎ウイルス(HCV)に感染していることから，肝硬変に対する肝移植後は，ウイルス性肝炎の再発が大きな問題となる図。B型肝炎再発に関しては，移植前からの核酸アナログ製剤投与と，術中・術後の高力価HBs抗体含有免疫グロブリン(hepatitis B immunoglobulin:HBIG)の投与によって再発を予防できるようになった。一方，C型肝炎に関してはいまだ標準的予防法はなく，その術後再発が大きな問題となっている。本項では，現在の肝移植後のウイルス肝炎対策の現状とその問題点について概説する。

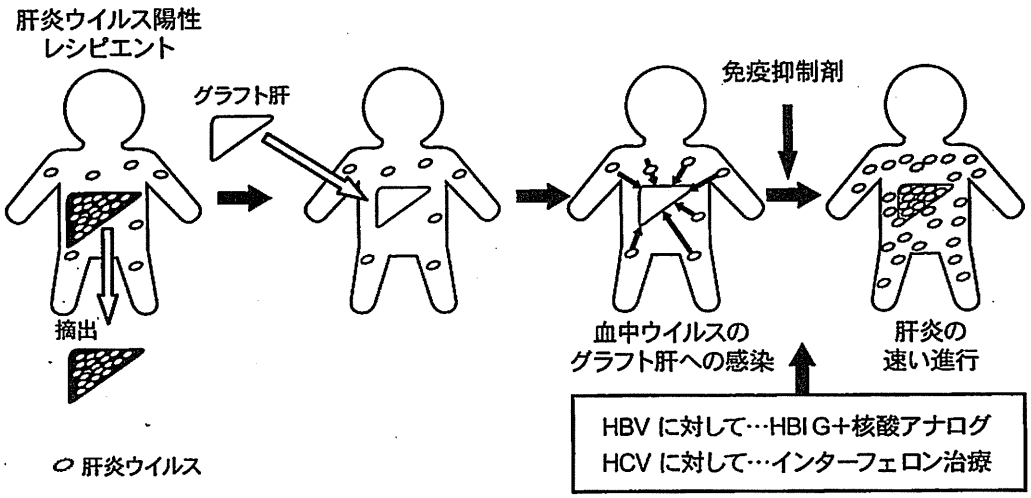

図 肝炎ウイルス陽性レシピエントにおける肝移植後の肝炎再発とその対策

肝炎ウイルス(HBVまたはHCV)陽性レシピエントに対する肝移植の際には，ウイルスが多量に存在する肝臓は摘出されるが，血中に残っているウイルスが移植されたグラフト肝に感染する。肝移植後には免疫抑制剤などの影響を受けてウイルスが増殖し，肝炎の進行が速いことが明らかになっている。この進行を予防するため，HBVに対してはHBIGと核酸アナログ製剤による予防策が，HCVに対してはインターフェロン治療が行われる。

HBIG：hepatitis B immunogloblin

1　B型肝炎ウイルス対策

1）肝移植後B型肝炎再発の特徴

HBs抗原陽性レシピエントに対して肝移植を行った場合，適切な予防処置を行わなければ，多くの症例で術後にB型肝炎の再発を認める。ヨーロッパにおけるHBs抗原陽性レシピエントに対する脳死肝移植後の報告では[1]，予防策を行わなかった場合の移植後HBs抗原の陽性化率は，B型肝硬変患者全体で67%であり，特に術前HBV DNAが陽性のレシピエントでは83%と高率にHBs抗原再陽性化を認める。血中のHBV DNAが陰性（測定感度以下）かつHBe抗原陰性であっても58%にHBs抗原陽性化を認めることから，血中HBVが減少していても依然として再感染可能なHBVが体内に存在していたことがわかる。一方，HBVによる急性肝不全例では，移植時に血中HBV DNAが陰性となっている症例が少なからず存在するため，移植後のHBs抗原陽性化率はB型慢性肝疾患と比べて低率であるが(17%)，現時点では移植後のグラフト肝への再感染の有無を移植時に予測することは困難である。これらの移植後B型肝炎再発症例は，術後のステロイドを含む免疫抑制剤の影響が一因となり急速に肝硬変へと進展することが知られており，その予後はきわめて不良であった。

2）肝移植後HBV再発予防法

これらのHBs抗原陽性レシピエントに対して，1990年代からB型肝炎再発予防法が試みられるようになった[2]。まず，HBIGの単独投与またはラミブジン単独投与が試みられ，一定の予防効果は認めたものの，多くの報告で30～40%程度のB型肝炎再活性化を認めた。その後，ラミブジンとHBIGの併用療法が行われ，ほぼ全例でB型肝炎再活性化が予防できるようになり，B型肝疾患に対する肝移植後の予後は著明に改善した。現在では移植前に核酸アナログ製剤の内服を開始し，術中からHBIGを投与，術後は核酸アナログ製剤とHBIGの併用を行うのが標準的予防法となっている。この核酸アナログ製剤とHBIGを併用したHBV対策により，術前のHBV DNA量に関係なく移植後のB型肝炎の再発は0～10%ときわめて低く抑えることができるようになった。そのため，HBs抗原陽性レシピエントに対する肝移植後の予後は他の疾患と比較して良好となっている。

3）問題点

上記の予防法にて，肝移植後のB型肝炎再発はほぼ全例で予防できるようになったが，依然いくつかの問題が残されている。1つはHBIG投与の経済的負担の問題である。2008年2月より肝移植後のB型肝炎再発・発症予防のためのHBIG投与が保険適用となり，以前よりは軽減されたものの，HBIGは高価であり長期間の投与は経済的負担が大きい。さらに，ヒト血液を原料とするため，供給量に限界もある。この問題に対し，B型肝炎ワクチン投与により能動免疫を誘導することによりHBIGの減量や中止の試みがなされている。また，ラミブジンに対する耐性変異株（YMDD変異株）の出現とそれに伴う肝炎再発の問題があり，実際に移植後ラミブジンの継続により10～20%

の頻度でYMDD変異株が出現する。この場合，アデホビル(adefovir dipivoxil)の追加が有効である。また，ラミブジンの代わりにエンテカビルを使用することによって変異株出現率は低下すると予想される。ただし，エンテカビル耐性変異株やアデホビル耐性変異株，HBIGに対するHBs抗体エスケープ変異株の出現も報告されており，今後問題となる可能性が示唆される。さらに，HBIGやラミブジン，アデホビル，エンテカビルの長期投与に対する安全性の問題や，ラミブジン，アデホビル，エンテカビルの妊婦への投与時の胎児に対する影響の問題などが残されている。

2 C型肝炎ウイルス対策

1）肝移植後C型肝炎再発の特徴

肝移植後C型肝炎には以下の5つの特徴がある[3]。

(1) 高い再発率

HCV陽性レシピエントに対して肝移植を行った際には，HCVが多く存在する肝臓が取り出され，未感染の正常肝が移植されるが，血中に残存するウイルスがグラフト肝に再感染する図。現時点ではその再感染を予防する方法はなく，ほぼ全例で移植後血中HCV RNAが陽性となり，大部分の症例で組織学的にも肝炎の再発が確認される。

(2) 高ウイルス量

肝移植直後はHCV RNA量は一時的に低下するが，すぐに移植肝に再感染し，多くの例において移植後1カ月程度で血中HCV RNA量が移植前の10～100倍にまで増加することが知られている。

(3) 急速な肝硬変への進行

肝移植後C型肝炎は進行が速いことが知られており，10～25%の症例が移植後5年以内という短い期間で肝硬変に進行することが知られている。感染(肝移植)から肝硬変へと至るまでの平均期間は10年とされており，通常のC型肝炎の20～30年の経過と比較して明らかに短い期間で肝硬変に至る。

(4) 胆汁うっ滞

肝移植後には，fibrosing cholestatic hepatitis（FCH）と呼ばれる，特殊な病態を示すことがあり，注意が必要である[4]。FCHは免疫抑制下に生じる原因不明の胆汁うっ滞性の肝障害を示す病態であり，HCV陽性レシピエントに対する肝移植例の7～15%程度に認めるとされている。本症は一度発症すると3～6カ月の経過で肝不全に進行し，きわめて予後不良である。また，FCHと診断されなくても，肝移植後C型肝炎の特徴として，胆道系酵素(ALPとγ-GTP)の上昇を認めることが多いことも明らかになっている。

(5) 他の肝疾患の併存の可能性

肝移植後は拒絶反応や胆道系合併症，血管系合併症など多くの合併症が生じる可能性があり，これらが肝移植後C型肝炎の病態や治療に影響を与える。また，これらの疾患とC型肝炎再発との鑑別が臨床上困難な例が多いため，肝移植後C型肝炎再発の診断も非常に困難となる。

2）C型肝炎ウイルス対策

肝移植後C型肝炎の治療としては，通常のC型慢性肝炎の治療と同様にペグインターフェロンとリバビリンの併用療法が中心となる[3, 5]。投与量や投与期間については，通常のC型慢性肝炎に準じて行われることが多い。しかしながら，肝移植後C型肝炎症例は，前述のように高ウイルス量であること，移植前に治療が行われ無効例が多く，遺伝子型1b型が多いこと，高齢者が多いこと，血球低下例が多いこと，免疫抑制剤などの影響で腎障害や糖尿病の合併症が多いこと，拒絶や胆管合併症などの移植後合併症が併存する場合があること，などの理由から，治療抵抗例が多く有害事象の発生率も高い。これらの問題のため，肝移植後C型肝炎の治療成績は満足いくものではなく，HCVの排除に至る例は全体の30%程度にとどまっている[5]。さらに，ウイルス排除に至らない場合には，急速に肝硬変へと進行する場合がある。そのため，各施設で以下のようなさまざまな工夫がなされている。

（1）治療開始時期の選択

肝移植後C型肝炎治療の特徴の一つとして，治療時期が選べるという点がある。これまでの報告から，大きく分けて，肝移植前，移植後早期（preemptive治療），肝炎再発後の3つの時期に治療が開始されている。

肝移植前治療については，ほとんどの症例が非代償性肝硬変であり，副作用の問題から術前の治療は困難である場合が多い。比較的肝予備能が保たれている肝細胞癌症例に対しては検討されるべき治療法であるが，治療期間中の肝細胞癌の進展が懸念される。移植後早期治療(preemptive治療)は，C型肝炎再発の有無にかかわらず，移植後数週間という早期から抗ウイルス治療を開始し，肝炎を予防しようとする試みである。術後早期の急速な進行やFCH発症の予防に有効である可能性がある一方で，移植後早期の全身状態の悪い状態での治療開始によって有害事象が増加する可能性もあり，今後，長期成績の比較などの検討が必要である。肝炎再発後の治療は，肝生検にて肝炎再発を確認してから，主にはF1以上の肝線維化を認め，門脈域の炎症を認める慢性肝炎期に治療を開始する治療法である。最も一般的な治療開始時期であり，欧米ではこの時期の治療が推奨されている。

（2）血球減少対策

肝移植後C型肝炎症例は，一度肝硬変を経ているために脾腫を伴っている症例がほとんどであり，多くの例で血球減少が持続している。その対策として，肝移植時に脾臓摘出術を行う施設が増えてきている。脾臓を摘出することによって，インターフェロン治療前に十分な血小板数が得られる。また，インターフェロン治療中の血球減少に対して，好中球低下にはG-CSF製剤が，貧血にはエリスロポエチン製剤が使用される場合があり，欧米では一般的な対策となっている。

（3）インターフェロン長期投与

肝移植後C型肝炎は難治性の場合が多いため，通常の投与期間ではウイルス排除を達成できず，治療終了後の再発率が高いことが明らかとなっている。そのため，治療期間を延長して治療を行う場合が多い。現在わが国の多くの施設で，血中HCV RNAが陰性化してから1年間治療を継続す

る個別化延長治療が行われている[6]。また，ウイルスを排除できない症例に対しては，インターフェロン治療を中止すると肝線維化が急速に進行することが多いため，インターフェロン治療を長期に渡って継続することが進行抑制につながると考えられる。わが国では多くの施設が長期継続投与を行っており，その線維化進行抑制効果が報告されている[7]。

3）問題点

肝移植後C型肝炎に対する治療の際には，有害事象の発生率が高く，治療の減量・中止例が多いことが問題となる。中でも，肝移植後症例に特徴的な2つの問題点を以下に示す。

（1）拒絶反応の誘発

インターフェロン治療によって拒絶反応が誘導される可能性が示唆されている。肝移植後に生じる拒絶反応は病理組織学的に大きく，急性（ならびに遅発性）細胞性拒絶反応，急性抗体関連拒絶反応，慢性拒絶反応，*de novo* 自己免疫性肝炎（特発性移植後肝炎）の4種類に分けられる。この中で，慢性拒絶と *de novo* 自己免疫性肝炎の2つが肝移植後C型肝炎に対するインターフェロン治療中に問題になることが報告されている[8, 9]。これらはいずれも放置すると肝不全へと進行する可能性が高いため，適切な免疫抑制剤の使用ならびに肝生検による早期診断，早期治療が必要である。

（2）免疫抑制剤の選択

シクロスポリンが *in vitro* で HCV の増殖を抑制することが報告され，C型肝炎再発に対する免疫抑制剤の影響が議論されている[10]。現在のところ，臨床的にはタクロリムスとシクロスポリンとの間でC型肝炎再発に対する影響に相違はないと考えられている[3]。一方，ステロイドパルス療法が肝移植後C型肝炎重症化のリスクファクターであるとする報告が多くなされている[3]。ステロイドを使用しないプロトコールによる免疫抑制が試みられているが，C型肝炎再発に対する影響についての結論は出ていない。前述したように，C型肝炎に対するインターフェロン治療によって拒絶反応が誘導される可能性もあるため，免疫抑制剤の安易な変更や減量は避けるべきであると考えられる。また，HCV 陽性例に拒絶反応を認めた際には，安易にステロイドパルス療法を行わず，可能であれば他の免疫抑制剤の増量で対処するほうが望ましいと考えられる。

おわりに

以上のように，肝移植後の大きな問題点として，肝炎ウイルス再発があり，その対策についてはいまだ多くの問題が残されている。今後，HCV または HBV 陽性肝疾患症例がますます増加することが予想される。手術手技や周術期管理の進歩により肝移植の短期予後は改善してきており，肝炎ウイルスのコントロールによる長期予後の改善が今後の重要な課題となるであろう。特に HCV 再発に関しては，治療開始の時期，適応，副作用の防止，免疫抑制剤の選択などの問題点を解決し，より有効な，副作用の少ない治療法を確立することが急務である。

（上田 佳秀）

文　献

1) Samuel D, Muller R, Alexander G, et al：Liver transplantation in European patients with the hepatitis B surface antigen. N Engl J Med 329：1842-1847, 1993
2) Papatheodoridis GV, Cholongitas E, Archimandritis AJ, et al：Current management of hepatitis B virus infection before and after liver transplantation. Liver Int 29:1294-1305, 2009
3) Watt K, Veldt B, Charlton M:A Practical guide to the management of HCV infection following liver transplantation. Am J Transplant 9：1707-1713, 2009
4) Schluger LK, Sheiner PA, Thung SN, et al:Severe recurrent cholestatic hepatitis C following orthotopic liver transplantation. Hepatology 23：971-976, 1996
5) Berenguer M：Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. J Hepatol 49:274-287, 2008
6) Ueda Y, Takada Y, Marusawa H, et al：Individualized extension of pegylated interferon plus ribavirin therapy for recurrent hepatitis C genotype 1b after living-donor liver transplantation. Transplantation 90：661-665, 2010
7) Ueda Y, Marusawa H, Kaido T, et al：Effect of maintenance therapy with low-dose peginterferon for recurrent hepatitis C after living donor liver transplantation. J Viral Hepat (in press).
8) Fernández I, Ulloa E, Colina F, et al:Incidence, risk factors, and outcome of chronic rejection during antiviral therapy for posttransplant recurrent hepatitis C. Liver Transpl 15：948-955, 2009
9) Berardi S, Lodato F, Gramenzi A, et al：High incidence of allograft dysfunction in liver transplanted patients treated with pegylated-interferon alpha-2b and ribavirin for hepatitis C recurrence：possible de novo autoimmune hepatitis? Gut 56：237-242, 2007
10) Watashi K, Hijikata M, Hosaka M, et al:Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. Hepatology 38：1282-1288, 2003

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ORIGINAL ARTICLE

Evaluation of long-term entecavir treatment in stable chronic hepatitis B patients switched from lamivudine therapy

Tatsuya Ide · Michio Sata · Kazuaki Chayama · Michiko Shindo · Joji Toyota · Satoshi Mochida · Eiichi Tomita · Hiromitsu Kumada · Gotaro Yamada · Hiroshi Yatsuhashi · Norio Hayashi · Hiroki Ishikawa · Taku Seriu · Masao Omata

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Abstract

Purpose Current Japanese guidelines recommend that patients should be switched from lamivudine to entecavir when they meet certain criteria. This analysis examines the efficacy and safety of long-term entecavir therapy in patients who were switched to entecavir after 24 weeks' lamivudine therapy in Japanese studies ETV-047 and ETV-060.

Methods The Phase II Japanese study ETV-047 assessed the efficacy of different entecavir doses when compared with lamivudine. A total of 33 Japanese patients who received lamivudine 100 mg daily in ETV-047 entered the open-label rollover study ETV-060 and subsequently received treatment with entecavir 0.5 mg daily. Hepatitis B virus (HBV) DNA suppression, alanine aminotransferase (ALT) normalization, hepatitis B e antigen (HBeAg) seroconversion, and resistance were evaluated among patients with available samples for up to 96 weeks. Safety was assessed throughout the treatment period.

Results After 96 weeks of entecavir therapy in ETV-060, 90% of patients achieved HBV DNA <400 copies/mL as compared to 21% of patients who completed 24 weeks of lamivudine therapy in ETV-047. Increasing proportions of patients achieved ALT normalization and HBeAg seroconversion following long-term entecavir treatment. No patients experienced virologic breakthrough, and substitutions associated with entecavir resistance were not

T. Ide (✉) · M. Sata
Division of Gastroenterology, Department of Medicine,
Kurume University School of Medicine, Fukuoka, Japan
e-mail: ide@med.kurume-u.ac.jp

K. Chayama
Department of Medicine and Molecular Science,
Graduate School of Biomedical Sciences,
Hiroshima University, Hiroshima, Japan

M. Shindo
Division of Liver Disease, Department of Internal Medicine,
Akashi Municipal Hospital, Akashi, Hyogo, Japan

J. Toyota
Department of Gastroenterology,
Sapporo Kosei General Hospital, Hokkaido, Japan

S. Mochida
Department of Gastroenterology and Hepatology,
Saitama Medical University, Saitama, Japan

E. Tomita
Department of Gastroenterology,
Gifu Municipal Hospital, Gifu, Japan

H. Kumada
Department of Hepatology, Toranomon Hospital, Tokyo, Japan

G. Yamada
Department of Internal Medicine, Center for Liver Diseases,
Kawasaki Medical School, Kawasaki Hospital, Okayama, Japan

H. Yatsuhashi
Clinical Research Center, National Hospital Organization
Nagasaki Medical Center, Nagasaki, Japan

N. Hayashi
Department of Gastroenterology and Hepatology,
Osaka University Graduate School of Medicine, Osaka, Japan

H. Ishikawa · T. Seriu
Research and Development, Bristol-Myers K.K, Tokyo, Japan

M. Omata
Department of Gastroenterology, Graduate School of Medicine,
University of Tokyo, Tokyo, Japan

observed in patients with detectable HBV DNA. Entecavir was well tolerated during long-term treatment.

Conclusions Switching lamivudine-treated patients with chronic hepatitis B to entecavir results in increased virologic suppression with no evidence of resistance through 2 years of entecavir therapy. These findings support recommendations in the current Japanese treatment guidelines that stable lamivudine patients should be switched to entecavir.

Keywords Japanese · Chronic hepatitis B · Entecavir · Lamivudine · Switch

Introduction

Chronic hepatitis B virus (HBV) infection affects more than 350 million people worldwide, and is a leading cause of liver-related mortality [1]. Although Japan has one of the lowest prevalence rates for chronic hepatitis B (CHB) (0.8%) among Asian countries, it is still estimated that over 1 million people are chronically infected with HBV [2]. These individuals are at an increased risk of developing cirrhosis, liver failure or hepatocellular carcinoma (HCC) [3].

Lamivudine was the first nucleoside analog introduced for the treatment of CHB. In clinical trials, it demonstrated superior efficacy to placebo for HBV DNA suppression, alanine aminotransferase (ALT) normalization and hepatitis B e antigen (HBeAg) seroconversion [4, 5]. However, a major limitation of lamivudine therapy is the development of resistance, which occurs in up to 70% of patients through 4 years of therapy [6]. Entecavir is a potent inhibitor of HBV replication [7]. In global Phase III studies, entecavir demonstrated superior histologic, virologic and biochemical responses when compared with lamivudine in nucleoside-naïve patients and lamivudine-refractory patients at 48 weeks [8–10]. In the Japanese Phase II study ETV-047, treatment with entecavir resulted in a superior reduction in HBV DNA as compared to lamivudine [11]. In contrast to lamivudine, entecavir has been shown to have a high genetic barrier to resistance; the cumulative probability of resistance through 5 years of treatment has been reported to be 1.2% [12]. The genetic barrier is lower in patients who are infected with lamivudine-resistant HBV and consequently higher resistance rates are observed in this population with long-term treatment [12].

Current Japanese treatment guidelines recommend that all treatment-naïve CHB patients with ALT levels $\geq$31 IU/L should be treated, dependent on their viral load. The thresholds for treatment are HBV DNA ≥ 5 $\log_{10}$ copies/mL in HBeAg-positive patients, ≥ 4 $\log_{10}$ copies/mL in HBeAg-negative patients, and ≥ 3 $\log_{10}$ copies/mL in cirrhotic patients [13]. Lamivudine, adefovir, and entecavir are currently approved for the treatment of CHB in Japan. Entecavir 0.5 mg once daily is the first choice therapy for treatment-naïve HBeAg-positive and negative patients aged 35 years or older. In treatment-naïve patients <35 years, the guidelines recommend treating first with interferon for HBeAg-positive patients, and treating HBeAg-negative patients with HBV DNA ≥ 7 $\log_{10}$ copies/mL with entecavir until undetectable HBV DNA is achieved, followed by a combination of entecavir and interferon for 4 weeks, and finally interferon monotherapy for 20 weeks. HBeAg-negative patients with HBV DNA <7 $\log_{10}$ copies/mL should be monitored or can receive interferon therapy. For patients who are lamivudine experienced, but not necessarily resistant, the guidelines also recommend that patients can be switched to entecavir 0.5 mg daily if they have received lamivudine therapy, and have HBV DNA <2.1 $\log_{10}$ copies/mL. Patients with HBV DNA ≥ 2.1 $\log_{10}$ copies/mL can also be switched to entecavir 0.5 mg once daily if they do not have viral breakthrough. Limited data on the efficacy of entecavir in this patient population are available; however, the design of the Japanese study ETV-047 and the rollover study ETV-060 presents an opportunity to assess the efficacy of this treatment option. This report examines the long-term efficacy, safety and resistance of entecavir 0.5 mg daily among patients who were directly switched from lamivudine following 24 weeks' treatment in ETV-047.

Materials and methods

Study population

Study ETV-047 was a Phase II, randomized, double-blind study conducted to evaluate the dose–response relationship of entecavir and compare the antiviral activity and safety of entecavir to lamivudine in Japanese patients with CHB. In ETV-047, 137 patients were randomized to receive one of three entecavir doses [0.01 mg ($n = 35$), 0.1 mg ($n = 34$) or 0.5 mg ($n = 34$), once daily] or lamivudine [100 mg ($n = 34$), once daily] for 24 weeks. The study design and complete inclusion criteria have been described previously [11]. Briefly, eligible patients had HBeAg-positive or -negative CHB with compensated liver disease, HBV DNA ≥ 7.6 $\log_{10}$ copies/mL by PCR assay, <12 weeks' prior therapy with anti-HBV nucleoside analogs and ALT levels 1.25–10 × upper limit of normal (ULN). After completion of treatment in ETV-047, all patients were eligible to enroll immediately in the rollover study ETV-060, with no gap in dosing.

The rollover study ETV-060 was designed to provide open-label entecavir for patients who had completed therapy in the Japanese Phase II program. Patients who completed 24 weeks of treatment in ETV-047 enrolled in ETV-060 and received 0.5 mg entecavir once daily. After 96 weeks of treatment in study ETV-060, patients could complete the study and were eligible to receive commercially available entecavir, which was approved by Japanese health authorities while study ETV-060 was ongoing.

The current analysis describes results for a subset of 33 patients who received lamivudine for 24 weeks in ETV-047 and entecavir 0.5 mg once daily for up to 96 weeks in ETV-060.

Efficacy analyses

Efficacy assessments evaluated the proportions of patients who had available samples (non-completer = missing) every 24 weeks through 120 weeks' treatment. Efficacy end points assessed included HBV DNA <400 copies/mL by PCR assay, ALT normalization (≤1.0 × ULN), HBeAg seroconversion among patients who were HBeAg-positive at baseline, and hepatitis B surface antigen (HBsAg) loss. Serum HBV DNA was determined by Roche Amplicor® PCR assay (Roche Diagnostics K.K., Tokyo, Japan; limit of quantification = 400 copies/mL) in a central laboratory. Clinical laboratory tests, PCR assays for HBV DNA, and serologic tests for HBV were performed at SRL, Inc. (Tokyo, Japan), the central clinical laboratory designated by the trial sponsor. On-treatment testing for resistance was carried out using a direct-sequencing PCR method.

Safety analyses

Safety analyses include the incidence of adverse events, serious adverse events, laboratory abnormalities, and discontinuations due to adverse events on-treatment throughout treatment in study ETV-060. On-treatment ALT flares were defined as ALT >2 × baseline and >10 × ULN.

Resistance analysis

Resistance testing was performed using a direct-sequencing PCR method. Paired samples from all patients with HBV DNA ≥400 copies/mL were analyzed for substitutions associated with entecavir or lamivudine resistance at week 96 (72 weeks of entecavir therapy) or week 120 (96 weeks of entecavir therapy). Patients who discontinued therapy prior to week 120 had their last on-treatment sample analyzed. All patients with virologic breakthrough (≥1 $\log_{10}$ increase from nadir on two consecutive measurements) were also tested for resistance.

Results

Study population

Of the 34 patients in ETV-047 who received treatment with lamivudine 100 mg once daily for 24 weeks, 33 entered ETV-060 and received treatment with entecavir 0.5 mg once daily. Two patients discontinued treatment during ETV-060: one due to an adverse event (depression) and the other due to insufficient effect. In addition, one patient completed treatment at week 76 (52 weeks of entecavir therapy) after meeting the criteria for protocol-defined complete response (undetectable HBV DNA by PCR assay, undetectable HBeAg and normal serum ALT).

Baseline demographic and disease characteristics for the switch cohort are presented in Table 1. The majority of patients (82%) in the cohort were male with a mean age of 43 years. The mean duration of entecavir therapy was 105.9 weeks (range 25–141 weeks). Baseline mean HBV DNA and ALT levels were 7.9 $\log_{10}$ copies/mL and 184 IU/L, respectively. Ninety-one percent of patients were HBeAg-positive and 88% had HBV genotype C infection.

Virologic end points

After completion of 24 weeks of lamivudine treatment in ETV-047, 21% (7/33) of patients in the switch cohort had achieved HBV DNA <400 copies/mL (Fig. 1). Following the switch to entecavir, the proportion of patients achieving HBV DNA <400 copies/mL increased to 82% (27/33) by week 48 (24 weeks of entecavir therapy). Viral suppression

Table 1 Baseline (pretreatment) demographics and disease characteristics: switch cohort

Characteristic	ETV-047/-60 lamivudine to entecavir switch cohort (n = 33)
Age, mean (years)	42.7
Male, n (%)	27 (82)
Ethnicity Japanese, n (%)	33 (100)
Entecavir treatment periods, mean (range) (weeks)	105.9 (25–141)
HbeAg-positive, n (%)	30 (91)
HBV DNA by PCR, mean $\log_{10}$ copies/mL (SD)	7.9 (0.80)
ALT (IU/L), mean (SD)	184.8 (132.9)
HBV genotype, n (%)	
A	2 (6)
B	2 (6)
C	29 (88)
Others	0

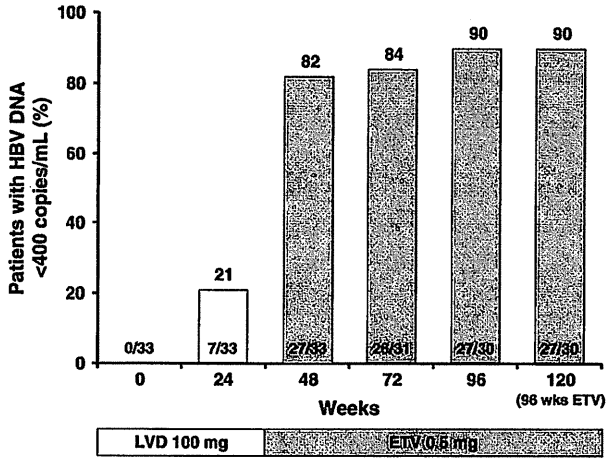

Fig. 1 Proportion of patients with HBV DNA <400 copies/mL through 120 weeks of therapy (ETV-047 to ETV-060). *Denominators* represent patients with available samples. *ETV* entecavir, *HBV* hepatitis B virus, *LVD* lamivudine

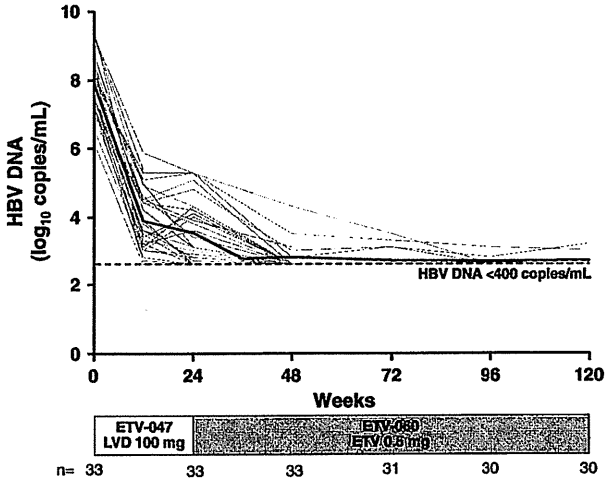

Fig. 2 HBV DNA suppression through week 120 (96 weeks of entecavir therapy). Individual patient HBV DNA profiles are plotted in *gray*. Mean HBV DNA levels are represented by the *solid black line*. *ETV* entecavir, *HBV* hepatitis B virus, *LVD* lamivudine

was maintained with longer entecavir treatment, with 84% (26/31) and 90% (27/30) achieving HBV DNA <400 copies/mL at weeks 72 and 120, respectively (48 and 96 weeks of entecavir therapy). Mean HBV DNA levels decreased from a baseline of 7.90 to 3.52 $\log_{10}$ copies/mL after 24 weeks of lamivudine therapy in ETV-047, and reached 2.69 $\log_{10}$ copies/mL after 96 weeks of entecavir therapy in ETV-060 (week 120; Fig. 2). No viral breakthrough was observed during entecavir therapy.

Biochemical end points

ALT normalization (≤1.0 × ULN) was demonstrated in 76% (25/33) of patients after 24 weeks of lamivudine therapy in

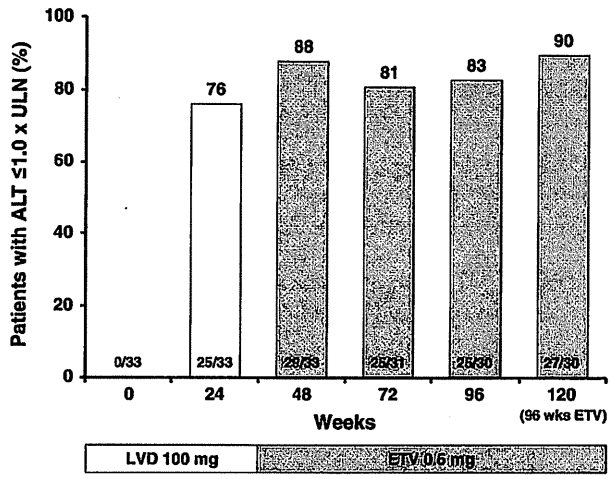

Fig. 3 Proportion of patients with ALT normalization (≤1.0 × ULN) through 120 weeks of therapy (ETV-047 to ETV-060). *Denominators* represent patients with available samples. *ALT* alanine aminotransferase, *ETV* entecavir; *LVD* lamivudine, *ULN* upper limit of normal

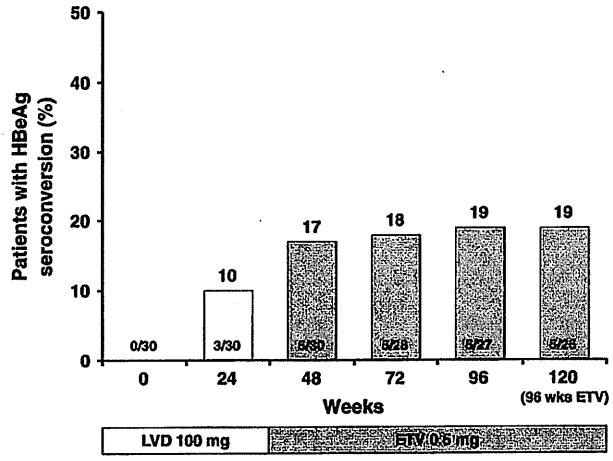

Fig. 4 Proportion of patients with HBeAg seroconversion through 120 weeks of therapy (ETV-047 to ETV-060). *Denominators* represent patients with available samples among the 30 patients HBeAg-positive at baseline. *ETV* entecavir, *HBeAg* hepatitis B e antigen, *LVD* lamivudine

ETV-047 (Fig. 3). Following treatment with entecavir in ETV-060, ALT normalization was maintained in 90% (27/30) of patients achieving this end point by week 120. Minor fluctuations in the proportion of patients achieving ALT normalization were attributed to patients discontinuing entecavir therapy during the course of study ETV-060.

Serologic end points

HBeAg seroconversion was assessed among the 30 patients in the switch cohort who were HBeAg-positive at baseline in ETV-047 (Table 1; Fig. 4). Three patients (10%)

achieved HBeAg seroconversion during the initial 24-week lamivudine treatment period in ETV-047 (Fig. 4). Following switch to entecavir in ETV-060, two additional patients developed HBeAg seroconversion by week 120 (96 weeks of entecavir therapy). None of the patients in the switch cohort experienced HBsAg loss during treatment in ETV-047 or ETV-060.

Resistance

Four of the 33 patients who received entecavir therapy in ETV-060 had HBV DNA ≥400 copies/mL either at treatment discontinuation or at week 120. One patient discontinued therapy at week 68 (44 weeks of entecavir therapy) due to insufficient effect. HBV DNA prior to treatment discontinuation was 3.1 $\log_{10}$ copies/mL, however, resistance testing revealed no substitutions associated with entecavir resistance. The remaining three patients had HBV DNA ≥400 copies/mL at weeks 96 and 120; however, only two patients had samples available for testing. Neither patient's samples had substitutions associated with entecavir or lamivudine resistance either at weeks 96 or 120.

Safety

Entecavir was well tolerated during long-term treatment and the safety profile of patients in the switch cohort was consistent with that previously reported for patients who received continuous entecavir therapy in studies ETV-047 and ETV-060 (Table 2). Serious adverse events (Meniere's disease, subcutaneous abscess and ALT flare) were reported in three patients (9.1%). The most frequently reported adverse events during treatment in ETV-060, occurring in ≥10% of patients, were nasopharyngitis (76%), diarrhea (21%), back pain (18%), influenza (18%), and allergic rhinitis (15%). One patient discontinued entecavir therapy due to depression, which the investigator considered was possibly related to entecavir therapy. An ALT flare (ALT >2 × baseline and >10 × ULN) occurred in one patient at week 18, and was judged a serious adverse event by the investigator, but was not associated with a change in HBV DNA. No deaths were reported during the study.

Table 2 Summary of safety in ETV-060: switch cohort

On-treatment in ETV-060	Patients, *n* (%)
Any adverse events	33 (100)
Clinical adverse events	33 (100)
Laboratory adverse events	33 (100)
Grade 3/4 clinical adverse event	1 (3)
Grade 3/4 laboratory adverse event	5 (15)
Clinical serious adverse event[a]	3 (9)
Discontinuations due to adverse events	1 (3)
Deaths	0
ALT flares[b]	1 (3)

[a] Including ALT flares

[b] ALT >2 × baseline and >10 × ULN

Discussion

Profound long-term suppression of HBV DNA is required for patients to meet the goals of CHB therapy, which are to prevent cirrhosis, hepatic failure, HCC and liver-related death [14–16]. A major concern with long-term therapy is the increasing risk of selecting resistance mutations, especially for therapies with a low-genetic barrier to resistance, such as lamivudine. The current analysis presents results for a cohort of Japanese patients who were switched directly from lamivudine to long-term entecavir therapy. The results show that this switch cohort achieved additional HBV DNA suppression after the switch to entecavir. The proportion of patients with HBV DNA <400 copies/mL increased from 21% after 24 weeks of lamivudine treatment to 82% following an additional 24 weeks of entecavir treatment. Mean HBV DNA decreased from 3.52 $\log_{10}$ copies/mL at week 24 to 2.80 $\log_{10}$ copies/mL at week 48. Rates of HBV DNA suppression were maintained in this cohort, with 90% of patients achieving HBV DNA <400 copies/mL through 96 weeks of entecavir therapy (week 120). These results are comparable to those achieved by the cohort of patients who received entecavir 0.5 mg once daily in the Japanese Phase II studies and the rollover study ETV-060 [17]. At baseline in ETV-060, 56% of this cohort had achieved HBV DNA <400 copies/mL, increasing to 83% through 96 weeks of entecavir therapy. Among patients with abnormal ALT levels at ETV-060 baseline, 88% of patients in the entecavir 0.5 mg cohort achieved normalized ALT levels at week 96 as compared to 90% of patients in the switch cohort. Rates of HBeAg seroconversion at week 96 in ETV-060 were also similar (20 vs. 19%, respectively). These rates of viral suppression also show comparison favorably to those reported for the global nucleoside-naïve cohorts treated for a similar period of time [18, 19]. The potent antiviral activity of entecavir and its high genetic barrier to resistance is expected to minimize the potential for resistance in the switch cohort, allowing long-term therapy for patients. Liver biopsies were not obtained from patients in the switch cohort; however, the histologic benefits of long-term entecavir therapy have been recently reported for a cohort of naïve Japanese patients in the ETV-060 rollover study [20]. Following treatment with entecavir 0.5 mg daily for 3 years, all patients experienced histologic improvement and 57% experienced improvement in fibrosis score. In

addition, the results from a separate global study have confirmed the histologic benefits of long-term entecavir treatment [21].

Previous Japanese (ETV-052/-060) and global (ETV-026) studies have examined the efficacy of entecavir in lamivudine-refractory patients. In these studies, entecavir demonstrated efficacy, with 54% of Japanese patients achieving HBV DNA <400 copies/mL through 3 years' treatment [10, 22]. However, as a result of the lower genetic barrier in these patients, a major drawback of entecavir therapy in this population is the development of resistance. The cumulative probabilities of genotypic entecavir resistance among lamivudine-refractory patients were 33% through 3 years' treatment in Japanese patients and 51% through 5 years' treatment among patients in the global cohort [12, 22]. In the current study, no entecavir- or lamivudine-associated resistance substitutions were detected after 96 weeks of entecavir treatment. However, in contrast to the previous studies where the majority of patients had high baseline HBV DNA and documented lamivudine resistance [10, 23], patients in the switch cohort received entecavir after achieving variable degrees of HBV DNA suppression with 24 weeks of lamivudine therapy. Therefore, the fact that no resistance has been observed in this cohort to date is not unexpected. This observation is consistent with an analysis of lamivudine-refractory patients enrolled in the worldwide lamivudine-refractory study ETV-026. Patients with baseline HBV DNA <7 $\log_{10}$ copies/mL had a higher probability of achieving HBV DNA <300 copies/mL as compared to those who had baseline HBV DNA ≥7 $\log_{10}$ copies/mL (73 vs. 16%) [24]. Furthermore, among the 42 entecavir-treated patients in ETV-026 who achieved HBV DNA <300 copies/mL through 96 weeks of therapy, only one patient subsequently developed entecavir resistance.

Current recommendations on the treatment of patients with documented lamivudine resistance suggest that patients should receive a second drug without cross resistance. The combination of lamivudine and adefovir has been shown to be superior to adefovir monotherapy for the treatment of lamivudine resistance, especially in preventing the selection of adefovir resistance [25–27]. Although only short-term clinical data are available for tenofovir, rates of viral suppression among lamivudine-experienced or -resistant patients who received tenofovir monotherapy do not differ significantly from those of treatment-naïve patients [28, 29]. Small studies have also shown pegylated interferon alpha-2a to be a safe and beneficial treatment option for lamivudine-experienced patients [30]. However, the treatment options for Japanese lamivudine-resistant patients are more limited, since neither tenofovir nor pegylated interferon alpha-2a are currently approved in Japan.

The Japanese guidelines recommend that patients with detectable YMDD mutations should receive treatment with a combination of lamivudine and adefovir [13]. However, the guidelines also allow patients who have received <3 years of lamivudine therapy, have HBV DNA <400 copies/mL, and no breakthrough hepatitis or YMDD mutations to switch directly to entecavir. The results presented in this analysis suggest that the strategy of switching to entecavir is an effective one that may avoid the additional cost and potential toxicity of combination treatment with lamivudine and adefovir. Among patients in the switch cohort, 96 weeks of entecavir treatment was well tolerated and the safety profile was comparable with previous experience in Japanese patients. One patient experienced an ALT flare (ALT >2 × baseline and >10 × ULN) 18 weeks after initiating entecavir, which was not associated with a change in HBV DNA. This low rate of ALT flares is consistent with previous findings and demonstrates that lamivudine-treated patients can be switched safely to entecavir with a minimal risk of such flares [10, 22].

In summary, the data from the switch cohort presented in this analysis demonstrate that CHB patients can be switched from lamivudine to long-term entecavir. The treatment with entecavir resulted in increased rates of virologic suppression with no evidence of resistance through 2 years of therapy. These findings support recommendations in the current Japanese treatment guidelines that patients on stable lamivudine therapy with no YMDD mutations should be switched to entecavir.

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References

1. Lavanchy D. Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. J Clin Virol 2005;34(Suppl 1):S1–S3
2. Merican I, Guan R, Amarapuka D, Alexander MJ, Chutaputti A, Chien RN, et al. Chronic hepatitis B virus infection in Asian countries. J Gastroenterol Hepatol 2000;15:1356–1361
3. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008;48:335–352

4. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, et al. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. N Engl J Med 1998;339:61–68
5. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Engl J Med 1999;341:1256–1263
6. Lok AS, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, et al. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. Gastroenterology 2003;125:1714–1722
7. Innaimo SF, Seifer M, Bisacchi GS, Standring DN, Zahler R, Colonno RJ. Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. Antimicrob Agents Chemother 1997;41:1444–1448
8. Chang TT, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001–1010
9. Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011–1020
10. Sherman M, Yurdaydin C, Sollano J, Silva M, Liaw YF, Cianciara J, et al. Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. Gastroenterology 2006;130:2039–2049
11. Shindo M, Chayama K, Kumada H, Yokosuka O, Sata M, Hayashi N, et al. Investigation of the antiviral activity, dose-response relationship and safety of entecavir following 24-week oral dosing in nucleoside-naive adult patients with chronic hepatitis B: a randomized double-blind Phase II clinical trial in Japanese patients. Hepatol Int 2009;3:445–452
12. Tenney DJ, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, et al. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naive patients is rare through 5 years of therapy. Hepatology 2009;49:1503–1514
13. Kumada H. Report of the research for therapy standardization of viral hepatitis including liver cirrhosis. The Research on Hepatitis, Health and Labor Science Research Grants: revised edition; 2010
14. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507–539
15. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. J Hepatol 2009;50:227–242
16. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int 2009;2:263–283
17. Yokosuka O, Takaguchi K, Fujioka S, Shindo M, Chayama K, Kobashi H, et al. Long-term use of entecavir in nucleoside-naïve Japanese patients with chronic hepatitis B infection. J Hepatol 2010 (in press)
18. Han SH, Chang TT, Chao YC, Yoon S, Gish RG, Cheinquer H, et al. Five years of continuous entecavir for nucleoside-naive HBeAg(+) chronic hepatitis B: results from study ETV-901. Hepatology 2008;48(Suppl 1):705A–706A
19. Shouval D, Lai CL, Chang TT, Gadano A, Wu SS, Halota W, et al. Three years of entecavir re-treatment of HBeAg(-) entecavir patients who previously discontinued entecavir therapy: results from study ETV-901. Hepatology 2008;48(Suppl 1):722A–723A
20. Yokosuka O, Kumada H, Toyota J, Takaguchi K, Kobashi H, Shindo M, et al. Three-year assessment of entecavir resistance in nucleoside-naïve and lamivudine-refractory Japanese patients with chronic hepatitis B. Hepatol Int 2008;2:A161
21. Liaw YF, Chang TT, Wu SS, Schiff ER, Han KH, Lai CL, et al. Long-term entecavir therapy results in reversal of fibrosis/cirrhosis and continued histologic improvement in patients with HBeAg(+) and (−) chronic hepatitis B: results from studies ETV-022, -027 and -901. Hepatology 2008;48(Suppl 1):706A
22. Izumi N, Kumada H, Toyota J, Yokosuka O, Kobashi H, Shindo M, et al. Efficacy and safety of 3 years treatment with entecavir in lamivudine-refractory Japanese chronic hepatitis B patients. Hepatol Int 2008;2:A186
23. Suzuki F, Toyoda J, Katano Y, Sata M, Moriyama M, Imazeki F, et al. Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: randomized controlled trial in Japanese patients. J Gastroenterol Hepatol 2008;23:1320–1326
24. Sherman M, Yurdaydin C, Simsek H, Silva M, Liaw YF, Rustgi VK, et al. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. Hepatology 2008;48:99–108
25. Peters MG, Hann HH, Martin P, Heathcote EJ, Buggisch P, Rubin R, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004;126:91–101
26. Rapti I, Dimou E, Mitsoula P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. Hepatology 2007;45:307–313
27. Lampertico P, Marzano A, Levrero M, Santantonio T, Di M, V, Brunetto M, et al. Adefovir and lamivudine combination therapy is superior to adefovir monotherapy for lamivudine-resistant patients with HBeAg-negative chronic hepatitis B. J Hepatol 2008;46 Suppl 1:S191
28. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med 2008;359:2442–2455
29. van Bommel F, de Man RA, Wedemeyer H, Deterding K, Petersen J, Buggisch P, et al. Long-term efficacy of tenofovir monotherapy for hepatitis B virus-monoinfected patients after failure of nucleoside/nucleotide analogues. Hepatology 2010;51:73–80
30. Leemans WF, Flink HJ, Janssen HL, Niesters HG, Schalm SW, de Man RA. The effect of pegylated interferon-alpha on the treatment of lamivudine resistant chronic HBeAg positive hepatitis B virus infection. J Hepatol 2006;44:507–511

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Original Article

Correlation of YMDD mutation and breakthrough hepatitis with hepatitis B virus DNA and serum ALT during lamivudine treatment

Mariko Kobayashi,[1] Fumitaka Suzuki,[2] Norio Akuta,[2] Hiromi Yatsuji,[2] Tetsuya Hosaka,[2] Hitomi Sezaki,[2] Masahiro Kobayashi,[2] Yusuke Kawamura,[2] Yoshiyuki Suzuki,[2] Yasuji Arase,[2] Kenji Ikeda,[2] Rie Mineta,[1] Satomi Iwasaki,[1] Sachiyo Watahiki[1] and Hiromitsu Kumada[2]

[1]*Research Institute for Hepatology, and* [2]*Department of Hepatology, Toranomon Hospital, Tokyo, Japan*

Aim: Continuous lamivudine treatment is associated with high frequency of drug resistance. We analyzed the incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant and breakthrough hepatitis (BTH) in hepatitis B virus (HBV) DNA positive patients receiving lamivudine for > 1 year and correlated it with HBV DNA and alanine aminotransferase (ALT) levels to evaluate if these measurements can provide a practical option for monitoring patients in clinical practice and define early switch from lamivudine therapy.

Methods: Of the 929 patients receiving lamivudine for > 1 year, 359 patients who maintained an ALT level of ≤ 40 IU/L during the course of lamivudine treatment were stratified into two groups based on the duration of lamivudine treatment – one receiving lamivudine for < 3 years and the other for ≥ 3 years.

Results: The incidence of YMDD motif in patients receiving lamivudine for < 3 years was 27% in patients with ALT ≤ 20 IU/L, 58% with ALT ≤ 30 IU/L, and 63% with ALT ≤ 40 IU/L, ($P = 0.002$). The corresponding incidence of BTH was 2%, 7%, and 48% ($P < 0.001$). The incidence of YMDD motif and BTH in these patients was 7% and 2% with HBV DNA < 2.6 (log copies/mL) and ALT ≤ 20 IU/L, while with ALT at 21–30, the YMDD motif mutant was 16% and BTH was 0%.

Conclusion: Correlation of ALT and HBV DNA levels with YMDD motif mutant and BTH indicates that these measurements can be used in clinical practice for deciding early switch from lamivudine to other suitable antiviral therapies.

Key words: alanine transaminase, breakthrough hepatitis, hepatitis B virus, lamivudine, mutation, viral DNA

INTRODUCTION

LAMIVUDINE HAS GAINED increasing popularity since its approval in 1998 for the treatment of chronic hepatitis B virus (CHBV).[1–4] Lamivudine blocks HBV replication, reduces HBV DNA levels, normalizes alanine aminotransferase (ALT) levels, thereby resulting in histological improvement of the liver.[5] It is a reverse transcriptase inhibitor that acts by competing with the natural polymerase substrate deoxycytidine triphosphate (dCTP) and thus inhibits the elongation of HBV DNA minus strand. It incorporates into the nascent DNA strand and thereby acts as a chain terminator. Although lamivudine is very effective in inhibiting viral replication, the incidence of resistance is high, with an estimated 14–32% of patients developing resistance after 1 year of treatment, 38% after 2 years of treatment, and 53–76% after 3 years of treatment.

Resistance to lamivudine, which increases over years is due to development of mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the DNA polymerase/reverse transcriptase, which is the main target of lamivudine.[4,6–9] This amino acid sequence in YMDD motif is predominantly involved in deoxynucleoside triphosphate (dNTP) binding in the catalytic site of the HBV DNA polymerase.

Correspondence: Dr Mariko Kobayashi, B.S., Research Institute for Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Kanagawa, Japan. Email: vj7m-kbys@asahi-net.or.jp

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Table 1 2007 Ministry of Health, Labour and Welfare of Japan guidelines for hepatits B virus (HBV)-positive patients for nucleoside analogue treatment for patients with chronic HBV receiving lamivudine therapy

Lamivudine therapy HBV DNA		< 3 years	≥ 3 years
Keep < 2.6 log copies/mL		Switch to entecavir 0.5 mg/day	Continue lamivudine 100 mg/day
≥ 2.6 log copies/mL	No BTH†	Switch to entecavir 0.5 mg/day	
	With BTH	Adefovir 10mg/day (duo therapy with lamivudine)	Adefovir 10 mg/day (duo therapy with lamivudine)

†After checking for absence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutation. BTH, breakthrough hepatitis.

Long-term lamivudine therapy is associated with amino acid substitutions mainly in the YMDD motif and also in the proximal FLLAQ (phenylalanine, leucine, alanine, glutamine) motif.[10] Common mutation may occur in the YMDD motif where the methionine residue is replaced either by valine (rtM204V) or isoleucine (rtM204I).[11] These amino acid substitutions form the basis of emergence of lamivudine-resistant strains of HBV and when these occur, the clinical condition may worsen, which is usually accompanied by increase in viral load and serum aminotransferase levels. YMDD mutants cause breakthrough hepatitis (BTH) and, therefore, require withdrawal or switch-over from lamivudine treatment. The American Association for the Study of Liver Diseases (AASLD) and the United States Algorithm for Management of Patients with Drug Resistance recommend either switching over to entecavir or adding adefovir in the event of lamivudine resistance.[12] The 2007 Japanese guidelines of the study group (Ministry of Health, Labour and Welfare of Japan)[13] on standardization of treatment for HBV positive patients for nucleoside analogue treatment for patients with CHBV receiving lamivudine therapy are explained below and also summarized in Table 1.

According to the 2007 guidelines for patients on lamivudine therapy, switching over criteria from lamivudine therapy has been changed from BTH to HBeAg status in patients maintaining HBV DNA copies ≥ 2.6 log copies/mL. Patients on lamivudine for < 3 years and maintaining HBV DNA copies ≥ 2.6 log copies/mL can be switched over to entecavir 0.5 mg/day if they are also HBeAg negative, whereas HBeAg-positive patients can be co-administered adefovir 10 mg/day in both the treatment duration groups (> 3 years or < 3 years).

Unfortunately, the cost of measuring HBV resistance to lamivudine by molecular methods is high and is not presently covered by Japanese reimbursement system in clinical practice. Development of HBV resistance to lamivudine is typically indicated by an increase in HBV DNA followed by an increase in serum ALT levels. Increase in HBV DNA represents active viral replication whereas serum ALT levels provide an indirect assessment of the degree of liver injury.[14]

Hence, in this study, we analyzed the correlation of the incidence of YMDD motif mutant and BTH with HBV DNA and serum ALT levels, either separately or together, in HBV DNA-positive patients who are treated with lamivudine for ≥ 1 year and who had maintained an ALT level of ≤ 40 IU/L until the development of BTH during the course of lamivudine treatment.

METHODS

Patients

THIS WAS A retrospective, nonrandomized study that enrolled 929 HBV DNA-positive-patients receiving 100 mg of lamivudine daily and followed up for a period of 1 year or longer between 1995 and 2006. Since long-term treatment with lamivudine was associated with a high frequency of YMDD motif mutant and BTH (BTH can be defined as abnormal variations in serum transaminase level due to YMDD motif mutant), we analyzed patients who had a possibility to switch to other nucleoside analogues. Patients ($n = 395$) with ALT ≤ 40 IU/L during follow-up (for 48 patients who developed BTH, data was used until 1 month before the patient developed BTH). Patients were not treated with either adefovir or entecavir during follow-up (for patients who used adefovir or entecavir because of BTH development, data was used until the point before the patient started adefovir or entecavir treatment). Patients were negative for anti-hepatitis C virus (HCV) (third-generation enzyme immunoassay; Chiron, Emerville, CA) and negative for HCV RNA with PCR (Amplicor; Roche Diagnostic Systems, Pleasanton, CA), did not have hepatocellular carcinoma, nore other forms of liver injury such as hemochromatosis, Wilson's disease,

primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease.

Informed consent was obtained from each patient included in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Patients were stratified into 2 groups based on the duration of lamivudine treatment – one receiving lamivudine for < 3 years ($n = 125$) and the other for ≥ 3 years ($n = 234$). In addition, we also analyzed patients based on their ALT level (IU/L) grouped into ≤20, 21–30, and 31–40, and HBV DNA (log copies/mL) divided into < 2.6, 2.6–5.0, and ≥ 5.1.

During treatment, patients were followed up each month for liver function and serum markers of HBV infection. The serum sample of the patients were collected and preserved at –80°C. All the collected samples up to this time period were analyzed for HBV DNA in June 2001. From July 2001, the serum samples were collected and analyzed once a month at the clinical treatment facility.

YMDD motif mutants were determined at the baseline and monitored at 6 months and during the study as well as at the development of breakthrough hepatitis. YMDD motif mutants were analyzed in the serum preserved at –80°C altogether.

Markers of HBV infection

The HBeAg was estimated by enzyme-linked immunosorbent assay (ELISA) (F-HBe; Sysmex, Kobe). HBV DNA was determined by PCR followed by hybridization (Amplicor HBV Monitor: Roche Molecular Systems, Branchburg, NJ), and the results were expressed in log copy per milliliter over a range of 2.6–7.6. The 6 major genotypes of HBV (A–F) were determined serologically by ELISA (HBV GENOTYPE EIA; Institute of Immunology) and the PCR-invader method with genotype-specific probes.[15] YMDD motif mutants were determined by PCR followed by restriction fragment length polymorphism (RFLP)[8] or enzyme-linked minisequence assay with commercial assay kits (PCR-ELMA; Genome Science).

Statistical analyses

Frequencies were compared between groups by the χ^2-test, Fisher's exact test, and HBV DNA values by Mann–Whitney *U*-test. Emergence of YMDD motif mutants and BTH were compared in the Kaplan–Meier life table by using the production limit method. A *P*-value < 0.05 was considered significant. Analyses of all data were performed with SAS 9.1.3.

RESULTS

DURING THE PERIOD of 12 years from 1995 to 2006, 929 HBV DNA-positive patients received 100 mg of lamivudine daily. From the total of 929 patients who received lamivudine for 1 year or more, 359 patients who maintained an ALT level of ≤ 40 IU/L were stratified based on the duration of lamivudine treatment and divided into 2 groups – one receiving lamivudine for < 3 years ($n = 125$) and the other for ≥ 3 years ($n = 234$). Demographic features and clinical background of the two study groups were uniformly matched with no significant differences in age, sex, serum transaminase levels, HBV DNA, hepatitis B e-antigen (HBeAg), and HBV genotype (Table 2). The median ALT values were 112 IU/L and 145 IU/L in both the groups, respectively, and the median HBV DNA level was identical at 6.1 log copies/mL in both the groups.

Incidence of YMDD motif mutant and BTH after lamivudine treatment for < 3 years

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine by ALT (IU/L) level was 27% in 53 patients maintaining an ALT level of ≤ 20 (group A), 58% in 46 patients maintaining an ALT level of ≤ 30 (group B); and 63% in 26 patients maintaining an ALT level of ≤ 40 (group C), with statistical differences among the 3 groups ($P = 0.002$). The incidence of BTH was 2% in group A, 7% in group B, and 48% in group C ($P < 0.001$). The lowest incidence of YMDD motif mutant and BTH was noted in patients with ALT level of ≤ 20 (IU/L) (Fig. 1a,b). Follow-up for patients who developed BTH was discontinued upon the detection of YMDD motif mutant.

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine based on the HBV DNA (log copies/mL) level was 28% in patients maintaining an HBV DNA level of < 2.6; 83% in patients maintaining an HBV DNA level of 2.6–5.0; and 100% in patients maintaining an HBV DNA level of ≥ 5.1, with significant differences among the 3 groups ($P < 0.001$). The incidence of BTH was 4%, 30%, and 40%, respectively, in patients with HBV DNA level of < 2.6, 2.6–5.0, and ≥ 5.1 log copies/mL ($P = 0.004$) (Fig. 2a,b). The lowest incidence of YMDD motif mutant and BTH was seen in patients maintaining an HBV DNA level of < 2.6 log

Table 2 Background of 359 patients using lamivudine treatment for ≥ 1 year at the start of lamivudine therapy

Factors	Duration of lamivudine therapy		
	< 3 years *n* = 125	≥ 3 years *n* = 234	Differences (*P*-value)
Age (years)	23–75 (43)†	18–76 (43)†	NS‡
Male	93 (73%)	182 (77.1%)	NS‡
HBV infection in mother	47 (37%)	82 (35%)	NS‡
Chronic hepatitis	109 (85%)	212 (90%)	NS‡
AST (IU/L)	15–866 (80)†	19–2593 (83)†	NS‡
ALT (IU/L)	11–2092 (112)†	14–2142 (145)†	NS‡
Total bilirubin (mg/dL)	0.2–3.8 (0.7)†	0.2–10.6 (0.7)†	NS‡
γ-GTP (IU/L)	16–440 (54)†	13–468 (65)†	NS‡
HBV DNA (log copy/mL)	<2.6–>7.6 (6.1)†	<2.6–>7.6 (6.1)†	NS‡
HBeAg	66(52%)	107 (45%)	NS‡
HBV genotype (A, B, C, ND)	4:15:98:8	5:21:207:1	NS‡

†Median value where indicated. ‡Not significant. ALT, alanine transaminase; AST, aspartate aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; γ-GTP, gamma glutamyl transferase.

copies/mL. The BTH incidence was particularly high in patients with an HBV DNA level of ≥ 5.1, which was 40% within 1 year.

The incidence of YMDD motif mutant within 3 years of treatment with lamivudine in patients based on both the ALT (IU/L) and HBV DNA (log copies/mL) level during the course of lamivudine treatment was evaluated (Table 3).

In patients maintaining HBV DNA < 2.6 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 7% and 2%, respectively. Whereas in patients with HBV DNA level of < 2.6 and ALT 21–30, the incidence of YMDD motif mutant was higher at 16% and BTH was 0%, and in patients with ALT 31–40, YMDD motif mutant and BTH was further higher at 42% and 17%, respectively.

In patients with HBV DNA level at 2.6–5.0 and ALT ≤ 20, the incidence of YMDD motif mutant was 33% in patients with 0% incidence of BTH. Nevertheless, in patients maintaining HBV DNA at 2.6–5.0 but with ALT 21–30, the incidence of YMDD motif mutant was 73% and BTH was 18%; whereas in patients with ALT 31–40, the incidence of YMDD motif mutant was 50% and BTH was 42%.

In patients maintaining HBV DNA ≥ 5.1 and ALT 31–40, both YMDD motif mutant and BTH was 100%.

Incidence of YMDD motif mutant and BTH after lamivudine treatment for ≥ 3 years

In patients treated with lamivudine for 3 years or more, the incidence of YMDD motif mutant by ALT (IU/L) level was 58% in 113 patients in group A, 60% in 84

Table 3 Incidences of tyrosine-methionine-aspartate-aspartate (YMDD) mutant and breakthrough hepatitis (BTH) by hepatits B virus (HBV) DNA and alanine transaminase (ALT) level in patients during lamivudine treatment for < 3 years (125 patients)

HBV DNA† (Amplicor: log copies/mL)	ALT level (IU/L)†					
	≤ 20		21–30		31–40	
	YMDD	BTH	YMDD	BTH	YMDD	BTH
< 2.6	3/41 (7%)	1/41 (2%)	5/32 (16%)	0/32 (0%)	5/12 (42%)	2/12 (17%)
2.6–5.0	4/12 (33%)	0/12 (0%)	8/11 (73%)	2/11 (18%)	6/12 (50%)	5/12 (42%)
≥ 5.1	0	0	3/3 (100%)	0/3 (0%)	2/2 (100%)	2/2 (100%)

†The HBV DNA and ALT levels are shown based on the treatment duration of lamivudine.

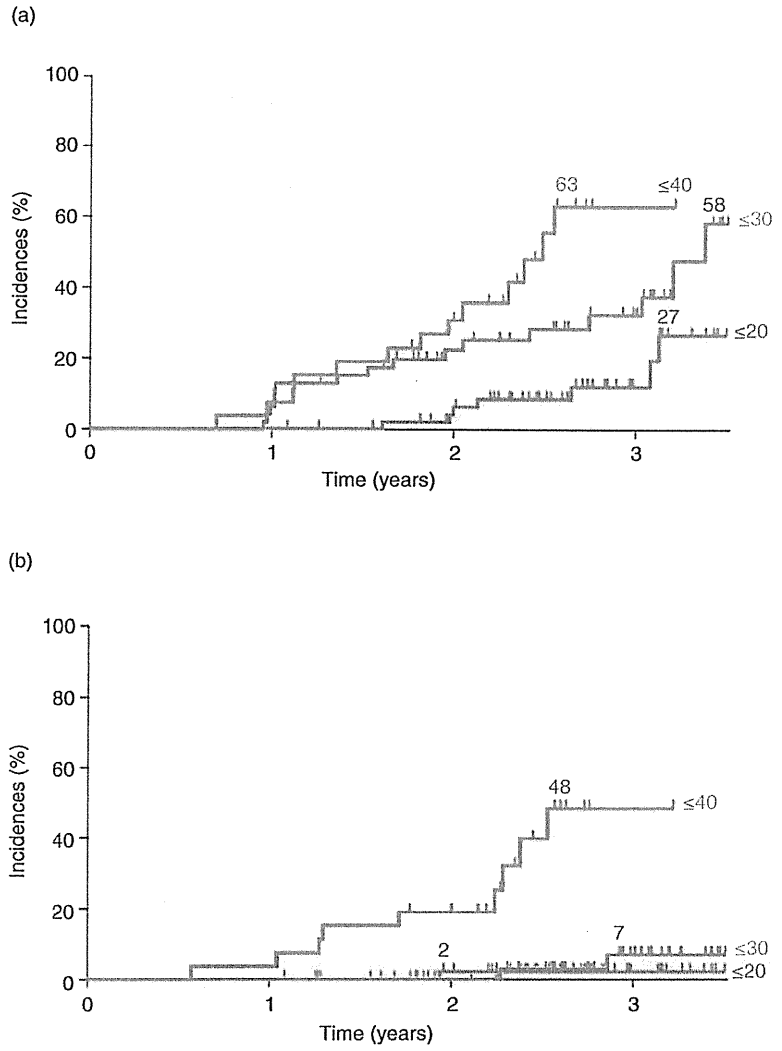

Figure 1 The incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant and breakthrough hepatitis was noted in patients with alanine aminotransferase level of ≤ 20 (IU/L) (a) Incidence of YMDD mutants over time ($P = 0.0017$). (b) Incidence of break throughhepatitis over time ($P < 0.0001$).

patients in group B, and 80% in 37 patients in group C ($P = 0.002$), and that of BTH in the corresponding groups was 7%, 14%, and 57% ($P < 0.001$) (Fig. 3a,b).

In patients treated with lamivudine for ≥ 3 years, the increased incidence of YMDD motif mutant by HBV DNA (log copies/mL) level was 65% in patients maintaining an HBV DNA level of < 2.6, 78% in patients maintaining an HBV DNA level of 2.6–5.0, and 92% in patients maintaining an HBV DNA level of ≥ 5.1, and that of BTH in the corresponding groups was 10%, 18%, and 77% ($P < 0.001$) (Fig. 4a,b).

The incidence of YMDD motif mutant in ≥ 3 years treatment with lamivudine in patients by both ALT (IU/L) and HBV DNA (log copies/mL) levels during the course of lamivudine treatment was also analyzed (Table 4).

In patients maintaining HBV DNA < 2.6 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 38% and 7%, respectively. At the same HBV DNA level of < 2.6 and ALT 21–30, the incidence of YMDD motif mutant was 48% and BTH was 8%; whereas at ALT 31–40, YMDD motif mutant was 36% and BTH was 9%.

In patients maintaining HBV DNA 2.6–5.0 and ALT ≤ 20, the incidence of YMDD motif mutant and BTH was 60% and 4%, respectively. At the same HBV DNA

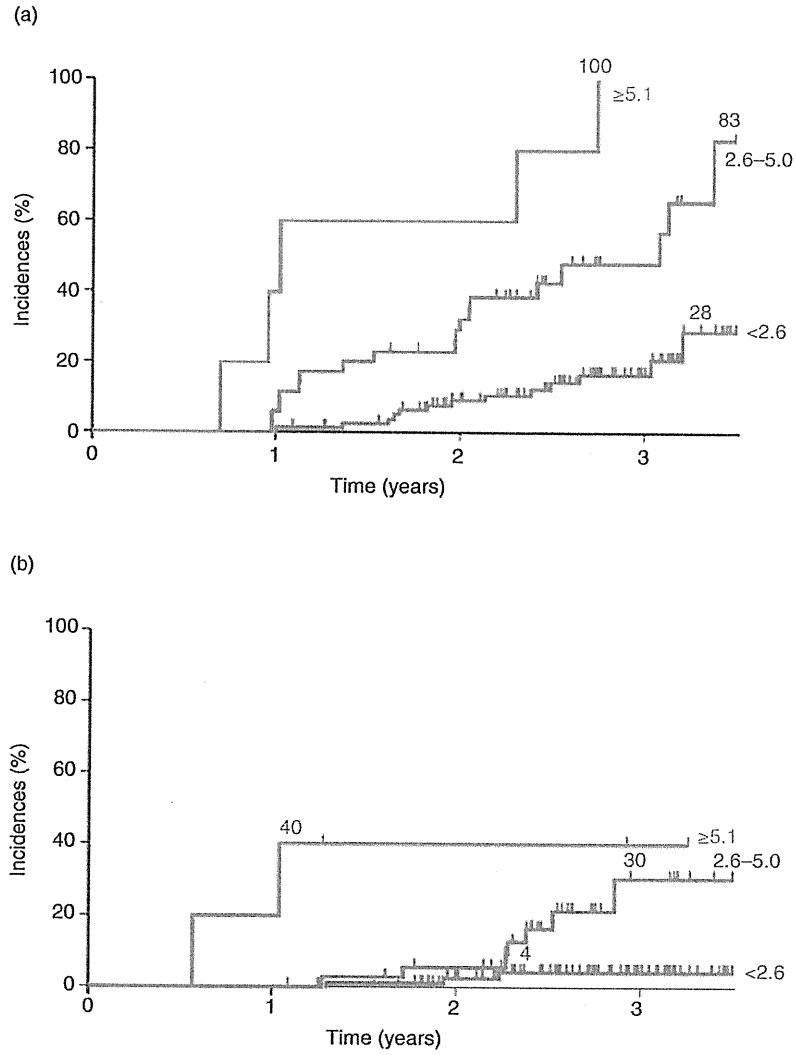

Figure 2 incidence of BTH was 4%, 30%, and 40%, respectively, in patients with HBV DNA level of < 2.6, 2.6–5.0, and ≥ 5.1 log copies/mL ($P = 0.004$). (a) Incidence of YMDD mutants over time ($P = 0.0001$). (b) Incidence of breakthrough hepatitis over time ($P < 0.0037$).

level, 2.6–5.0 and ALT 21–30, the incidence of YMDD motif mutant was 86% and BTH was 18%; whereas at ALT 31–40, YMDD motif mutant was 92% and BTH was 42%.

In patients maintaining HBV DNA ≥ 5.1 and ALT 31–40, YMDD motif mutant was 93% and BTH was 86%.

DISCUSSION

LONG-TERM THERAPY for CHBV can lead to the development of HBV drug-resistant mutants. Early detection of the YMDD motif mutants in lamivudine-treated patients and timely switch to other nucleoside analogues with low viral resistance is crucial to prevent viral and biochemical flares and ineffective therapeutic response. Although development of YMDD mutants results in decreased viral susceptibility to lamivudine, viral replication rate is lower in mutant strains than in wild type.[6]

Among the 359 patients who received lamivudine for > 1 year and maintained an ALT level of ≤ 40 IU/L, the rate of YMDD motif mutant was 11% (1 year), 29% (2 year), 42% (3 year), 49% (4 year) and 61% (5 year). BTH occurrences were 3% (1 year), 8% (2year), 13% (3 year), 15% (4 year) and 19% (5 year). The rate of

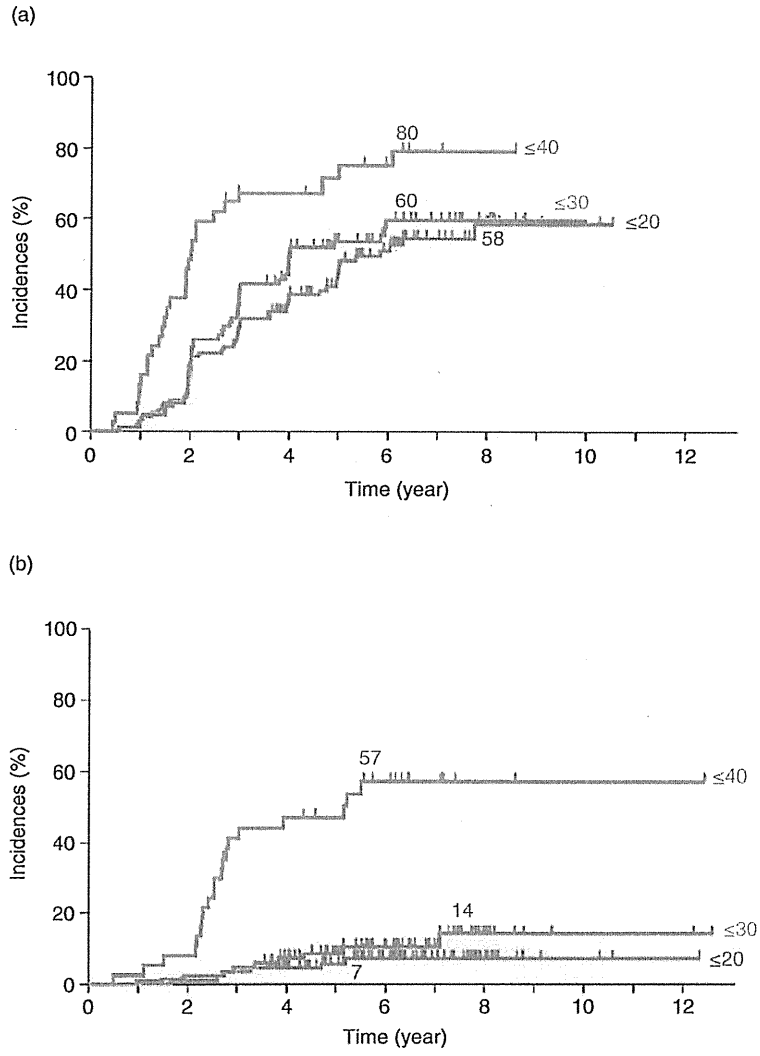

Figure 3 In patients treated with lamivudine for 3 years or more, the incidence of tyrosine-methionine-aspartate-aspartate (YMDD) motif mutant by alanine aminotransferase (IU/L) level was 58% in 113 patients in group A, 60% in 84 patients in group B, and 80% in 37 patients in group C ($P = 0.002$), and that of BTH in the corresponding groups was 7%, 14%, and 57% ($P < 0.001$). (a) Incidence of YMDD mutants over time ($P = 0.0015$). (b) Incidence of breakthrough hepatitis over time ($P < 0.0001$).

YMDD motif mutant and BTH were low after 3 or more years of treatment with lamivudine. Therefore, the year of switching treatment from lamivudine to other nucleic acid analogue will be at 3 years. Accordingly, in this study, we examined patients treated with lamivudine for < 3 and ≥ 3 years.

Among the patients treated with lamivudine for < 3 years, the lowest incidence of YMDD motif mutant and BTH was seen in patients with ALT < 20 IU/L maintaining HBV DNA level of 2.6–5.0. The other category for lowest incidence was in patients with ALT 21–30 IU/L and HBV DNA level of < 2.6 log copies/mL. In this study, within 3 years of treatment with lamivudine, the group of patients with the recommended HBV DNA (< 2.6 log copies/mL) and ALT maintained at 21–30 IU/L may be considered eligible to be switched to entecavir therapy as per Japanese guidelines. We, however, believe it is important to consider the prognosis for patients who are switched from lamivudine to entecavir. Similarly, in patients maintaining HBV DNA level in the range of 2.6–5.0 log copies/mL and ALT < 20 IU/L, switching to dual therapy with adefovir in combination with lamivudine depends on the related viral breakthrough. In a study by Li Zhou *et al.*,[16] some patients with YMDD motif mutants had significantly lower HBV DNA and ALT levels compared with baseline