characteristics	controls (n = 29)	HCV-infected patients (n = 21)	*p* value
sex, n (%)			> 0.999 †
male	16 (55.2)	13 (61.9)	
female	13 (44.8)	8 (38.1)	
age, years§	58.1 ± 10.8	52.2 ± 10.7	0.0632 ‡
HCV viral load¶, n (%)			
10^3	-	2 (6.9)	
10^4	-	2 (6.9)	
10^5	-	4 (13.8)	
10^6	-	10 (34.5)	
10^7	-	3 (10.3)	
HCV genotype, n (%)			
1b	-	13 (44.8)	
2a	-	7 (24.1)	
2b	-	1 (3.4)	
histology, n (%)			
CPH	-	5 (17.2)	
CAH	-	8 (27.6)	
LC	-	5 (17.2)	
NT	29 (100)	3 (10.3)	
virological responder, n (%)	-	8 (27.6)	
biochemical responder, n (%)	-	9 (31.0)	

§ Mean value ± SD
¶ The unit of HCV viral load is "copies per 50 μl of serum"
† Comparison by χ^2 test with Yate's compensation
‡ Comparison by unpaired student's *t*-test
CPH: chronic persistent hepatitis
CAH: chronic active hepatitis
LC: liver cirrhosis
NT: not tested

it was shown that IFN-α2 and IFN-β require a distinct intracytoplasmic region of the β_L subunit for their antiviral response, suggesting activation of a distinct signaling pathway by IFN-β [50]. In another report, it was shown that expression of CXCL11 (alias β-R1, I-TAC), a CXC chemokine ligand for CXCR3, was selectively induced by IFN-β, but not by IFN-α in a fibrosarcoma cell line [51, 52]. These findings suggested differences in biological activity between IFN-α and -β, and that it may be associated with the differences in the level of activity in IL-15 upregulation. We have also reported differences in clinical outcome between patients treated with IFN-α and -β. Sustained virological response was predicted by disappearance of HCV viremia early in the course of IFN administration in patients treated with IFN-α [53], but not in patients treated with IFN-β [11]. Moreover, by IFN-α treatment, the risk of HCC was significantly reduced in chronic hepatitis C patients with a biochemical response. In contrast, in patients treated with IFN-β, there was no difference in the incidence of HCC between patients with and without biochemical response, and there was a tendency for the risk to be reduced irrespective of the biochemical response of patients treated with IFN-β [14]. Thus, differences in biological activity between IFN-α and -β have been suggested by both basic and clinical studies, and these differences may be associated with the differences in the level of activity in IL-15 upregulation.

There was no significant difference in the serum IL-15 level of the patients treated with IFN-α and -β during the administration period. One possible explanation is that the administration routes are different for IFN-α and -β, i.e. intramuscular and intravenous injection, respectively. This difference would affect the drug concentration in tissues including liver. Another possibility is varied response to type I IFN among different cell types,

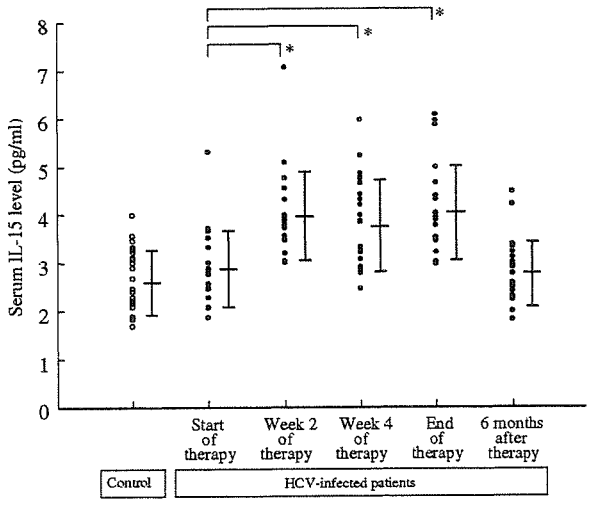

Fig. 5 The serum IL-15 level of IFN-treated patients with type C chronic liver disease and controls. The IL-15 level was quantified by ELISA. Unpaired Student's *t*-test was used to assess the statistical significance of differences in serum IL-15 levels between sera from controls and HCV-infected patients before treatment. Paired Student's *t*-test was used to compare serially assayed serum IL-15 of the HCV-infected patients. *Circles* indicate the individual serum IL-15 levels of the controls (*open circle*) and HCV-infected patients (*closed circle*). *Horizontal bars* show mean ± SD in each sample group. $*P < 0.0001$

or that only some cells or organs show differences in IL-15 expression response as between IFN-α and -β, resulting in the differences being overshadowed by other aspects of systemic response in vivo.

The present study provided the first evidence that type I IFN induces IL-15 production in vitro in HCC cell lines and also suggests a mechanism by which IL-15 production from non-immune cells mediates IFN-α/β-induced immune response. Our study also demonstrated that IFN-α and -β induce IL-15 production in human in vivo. These findings may help us better understand the immune regulatory mechanism of IFN-α/β and its implication in IL-15 expression. However, the precise roles of IFN-α/β are still unclear and further study is needed to clarify these issues. Such study will be useful for the development of selective and effective therapies for viral infection and cancer.

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Long-term lamivudine treatment for chronic hepatitis B in Japanese patients: A project of Kyushu University Liver Disease Study

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Abstract

AIM: To determine the efficacy of long-term lamivudine treatment of a large number of Japanese patients with chronic hepatitis B.

METHODS: In this retrospective, multi-center trial, 318 Japanese patients with chronic hepatitis B received 100 mg of lamivudine daily for up to 36 (median 21) mo. Virological response was a decline to a serum HBV DNA level less than 3.7 log copies/mL. Virological breakthrough was defined as the reappearance of a serum HBV DNA level to more than 10-fold the minimum during treatment.

RESULTS: Lamivudine produced virological response in 86.8% of the 318 patients at 6 mo, in 80.2% of 252 patients at 12 mo, in 69.2% of 133 patients at 24 mo, and in 53.6% of 28 patients at 36 mo. Forward stepwise logistic regression analysis showed an HBV DNA level less than 6.8 log copies/mL ($P<0.0001$), HBeAg negativity ($P<0.0001$), a platelet count of 100×10^9/L or more ($P=0.0162$) at baseline, and a decline of the HBV DNA level of more than 3.2 log copies/mL as compared with the baseline level at 3 mo after the start of treatment ($P=0.0003$) to be significantly associated with virological response. Among patients with a virological response, virological breakthrough was seen in 5.3% of 19 patients who responded virologically at 1 mo, in 20.7% of 203 patients at 3 mo, in 27.5% of 51 patients at 6 mo, in 33.3% of 12 patients at 9 mo, and in 100% of 3 patients at $\geqslant$15 mo. A virological breakthrough was found significantly more often in patients with delayed virological response.

CONCLUSION: Lamivudine treatment could suppress serum HBV DNA in most of the tested Japanese patients. Long-term efficacy might be seen in patients without HBeAg at baseline, in the absence of cirrhosis, and in patients with a decline in HBV DNA level soon after the start of treatment.

Key words: Hepatitis B virus; Lamivudine; HBeAg; Cirrhosis

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection affects 400 million people worldwide, three-quarters of whom reside in Asia[1]. The morbidity and mortality of chronic HBV infection are a major public health concern. Vertical transmission of HBV is the main cause of chronic HBV infection in the endemic areas of Asia. In Japan which is endemic for HBV infection, transmission can be easily prevented by vaccination of at risk-infants. The prevalence of hepatitis B surface antigen (HBsAg) carriage in the general population was reported to be less than 2%[2]. However, in areas of high HBV endemicity, persons with HBV-related cirrhosis have an approximately threefold higher risk of hepatocellular carcinoma (HCC) than those with chronic hepatitis but without cirrhosis and a 16-fold higher risk of HCC than the carriers in whom the virus is inactive[3]. Interferon-alpha (IFN-α) has been approved for the treatment of chronic hepatitis B[4], but it is poorly tolerated and effective in only 20%-30% of patients. Since the late 1990s, several studies have demonstrated the effectiveness of the initially available antiviral medicine, lamivudine, for patients with chronic hepatitis B: HBV DNA suppression, normalization of alanine aminotransferase (ALT), loss of hepatitis B e antigen (HBeAg). Moreover, studies have demonstrated the improvement of hepatic histology by the administration of lamivudine compared to placebo[5-8].

Lamivudine, an oral cytosine nucleoside analog, is the (-) - beta-enantiomer of 2',3'-dideoxy-3'-thiacytidine. HBV replicates through a pregenomic RNA intermediate. Lamivudine interferes with HBV reverse transcriptase (DNA polymerase) activity and causes chain termination of nascent viral DNA, leading to the inhibition of HBV replication[9]. Long-term treatment with lamivudine is not an option because it leads to drug resistance in most cases[10,11]. Lamivudine treatment, especially for chronic HBV-infected patients with cirrhosis, may also act as a bridge to more definitive treatments, such as liver transplantation. However, in several countries, including Japan, liver transplantation is not easily available because of insufficiency of donors, and even in other countries, many patients have to wait for long periods for transplantation. Although several non-Asian studies, from North America and Europe, have shown the efficacy of long-term use of lamivudine[12,13], few studies have assessed the efficacy of long-term lamivudine treatment of a large number of Japanese patients with chronic hepatitis B.

To acquire more data on these issues, 37 Japanese liver units involved in the management of HBV-related chronic liver diseases in Kyushu, Japan cooperated in this study. The objective of the present study was to analyze the results of long-term lamivudine administration for the suppression of HBV replication and the clinical outcomes of a large number of Japanese patients with chronic hepatitis B.

MATERIALS AND METHODS

Patients

This retrospective analysis encompassed 318 Japanese chronic hepatitis B patients (231 males and 87 females, mean age 47.8 years) on lamivudine monotherapy for up to 36 (median 21, range 9-36) mo. Clinical features from 403 HBsAg-positive patients with chronic liver diseases, who started lamivudine treatment between December 2000 and March 2004 in 37 Japanese liver units in Kyushu, were recorded in a centralized database. All patients were determined to be serum HBV DNA-positive via polymerase chain reaction (PCR) assay prior to treatment. The diagnosis of chronic hepatitis and cirrhosis was based on a liver biopsy in most patients, if unavailable, on clinical laboratory, and ultrasound data. Eighty five patients were excluded from the present analysis because of one or more of the following reasons: age below 18 years; positive for antibody to hepatitis C virus or human immunodeficiency virus type 1; diagnosis of HCC within 3 mo after enrolment; time of lamivudine treatment within 9 mo; or treatment with anticancer drugs or corticosteroid drugs for other malignancies, such as leukemia, lymphoma or autoimmune diseases. Because this was a retrospective analysis of treated patients, there were no predefined criteria for treatment withdrawal or combination treatments. Criteria for withdrawal and combination treatments after the start of the treatment were dependent upon the strategy used by the physician at each center. In the present study, follow-up was stopped for patients who discontinued lamivudine treatment or started receiving a combination treatment with IFN and lamivudine, or with adefovir dipivoxil and lamivudine.

Therapeutic protocol

The patients received lamivudine (Zeffix®, Glaxo Smith Kline, UK) orally in a single daily dose of 100 mg. Data concerning age, sex, history of prior IFN treatment, Child-Turcotte-Pugh (CTP) score, series of serum laboratory testing of ALT, total bilirubin, albumin, HBeAg, and HBV DNA level were collected. Also, we analyzed virological (time of virological response and virological breakthrough) and biological events (time of ALT normalization, ALT breakthrough, and hepatitis flare) during the observation period. The clinical events recorded were hepatic decompensation (ascites, portal hypertensive bleeding, and hepatic encephalopathy) and liver-related death during the study period.

Biochemical and virological measurement

Quantification of serum HBV DNA was performed at each center using one of the following commercial assays according to local availability: quantitative PCR assay (Amplicor HBV Monitor, Roche Diagnostics, Mannheim, Germany), over a detection range from 2.6 (corresponding to 400 copies/mL) to 7.5 log copies/mL; or transcription-mediated amplification and hybridization protect assay (TMA-HPA, Chugai Diagnostics, Tokyo, Japan), over a detection range from 3.7 log genome equivalents (LGE)/mL (corresponding to 5000 copies/mL) to 8.7 LGE/mL. A decline of the serum HBV DNA level to less than 3.7 log copies/mL during treatment was considered as a virological response. Virological breakthrough was defined as the reappearance of a serum HBV DNA level to more

than 10-fold the minimum during treatment. We analyzed whether or not an early decline of the HBV DNA level at 3 and 6 mo after the start of the treatment was related to virological response and breakthrough.

The serum ALT, bilirubin, and albumin levels were serially determined using the standard method every month before treatment and during the treatment. The upper normal limits for the ALT level were slightly different in each facility, ranging between 30 and 40 IU/mL. Normalization with an ALT level 667 or below during the treatment was considered as a biological response. A deterioration of ALT to an abnormal level after normalization during the treatment was considered as an ALT breakthrough. A deterioration of the ALT level more than 10 times the upper limit of normal (ULN) was considered as a hepatitis flare.

Statistical analysis

Categorical variables were analyzed using χ^2 test or Fisher's exact test. The Mann-Whitney *U*-test was also used to compare responders and non-responders with regard to various characteristics, when appropriate. The Cochran-Armitage's trend test was used to determine the relationship between the increases or decreases in the virological breakthrough rates of patients with virological response. Independent factors associated with responders were studied using forward stepwise logistic regression analysis of the following variables: age at the start of treatment, sex, history of prior IFN treatment, histological staging and grading, pretreatment laboratory data, serum pretreatment HBV DNA level, and the median declines of HBV DNA level at 3 and 6 mo after the start of the treatment. Forward stepwise logistic regression analysis was performed using a commercially available software package (BMDP Statistical Software Inc., Los Angeles, CA, USA) for the IBM 3090 system computer. The BMDP program LR was used to evaluate the relationship between the clinical features and SVR. Using this method, the most significant associated variable was entered into the model. After adjusting for that variable, the next most significant variable was added to the model. Two-tailed *P* values less than 0.05 were considered statistically significant.

RESULTS

Baseline assessment

The mean age and percentage over 35 years were significantly higher in patients with cirrhosis than in those without cirrhosis, while the mean ALT level, albumin, and platelet counts were significantly higher in patients without cirrhosis than in those with cirrhosis. No significant differences in sex distribution, total bilirubin, positivity of HBeAg, or HBV DNA level were observed between these groups (Table 1). This study consisted of 173 HBeAg-positive and 145 HBeAg-negative patients with a mean pretreatment HBV DNA level of 6.8±1.2 (median 7.0) log copies/mL. Concerning the relationship between HBeAg and HBV DNA level, the mean HBV DNA level was significantly higher in HBeAg-positive patients with (7.3±1.1 log copies/mL) and without cirrhosis (7.2±1.0 log copies/mL) as compared with HBeAg-negative patients with (6.3±1.2 log copies/mL) and without cirrhosis (6.2±1.1 log copies/mL) (both $P<0.0001$). No significant difference in HBV DNA level, even classified by HBeAg status, was observed between patients with and without cirrhosis.

Table 1 Baseline characteristics of 318 patients with chronic HBV infection treated with lamivudine (mean± SD)

Characteristics	Cirrhosis		*P*
	No *n* = 216	Yes *n* = 102	
Number of men (%)	154 (71.3)	77 (75.5)	0.5168
Age (yr)	45.0±11.1	53.7±9.7	<0.0001
Number with 35 yr old and over (%)	173 (80.1)	98 (96.1)	0.0003
ALT (IU/L)	320.9±503.3	101.5±95.4	<0.0001
Total bilirubin (mg/dL)	1.2±1.3	1.4±1.3	0.1880
Albumin (g/dL)	4.0±0.4	3.5±0.6	<0.0001
Platelet count (mean ± SD) (x10⁴/μL)	16.2±5.3	9.8±4.4	<0.0001
Number of HBeAg positivity (%)	119 (55.1)	54 (52.9)	0.8112
HBV-DNA[1]	6.8±1.3	6.6±1.2	0.1344
Lamivudine treatment (mo)	20.2±8.9	21.8±9.7	0.1429

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; SD, standard deviation [1]Logarithmic transformed copies/mL.

Virological and biological efficacy during lamivudine treatment period

In analyses of an early decline of HBV DNA level by lamivudine, the mean declines of all the studied patients were 3.2±1.2 (median 3.2) log copies/mL at 3 mo and 3.6±1.1 (median 3.8) log copies/mL at 6 mo after the start of treatment. During the treatment period of up to 36 (median 21) mo, a virological response was found in 90.6% (288/318) patients and ALT normalization was found in 86.2% (274/318) patients. Of the 288 with virological response, 255 (88.5%) had ALT normalization. Of the remaining 30 without virological response, 19 (63.3%), who had achieved virological suppression with a low HBV DNA level of 3.7-4.0 log copies/mL by lamivudine, had ALT normalization, but 11 (36.7%) had no ALT normalization and an HBV DNA level of more than 4.0 log copies/mL.

The mean pretreatment HBV DNA level was significantly lower in patients with virological response (6.6±1.2 log copies/mL) than those without virological response (7.7±0.7 log copies/mL) ($P<0.0001$). The frequency of pretreatment HBeAg positivity was significantly lower in patients with virological response (51.0%, 147/288) than those without virological response (86.7%, 26/30) ($P=0.0004$). No significant differences in sex distribution, age, ALT level, platelet count, presence of cirrhosis, or CTP score were found between the patients with and without virological response (Table 2).

Lamivudine suppressed serum HBV DNA to less than 3.7 log copies/mL in 69.2% patients at 3 mo, in 86.8% patients at 6 mo, in 80.2% patients at 12 mo, in 69.2% patients at 24 mo, and in 53.6% patients at 36 mo. The efficacy rate of virological response decreased with the length of the treatment period of patients who received lamivudine for over 6 mo (Figure 1).

Of the 288 patients with virological response, 224

Table 2 Virological response of 318 patients with chronic HBV infection treated with lamivudine (mean± SD)

Characteristics	Virological response		P
	No n = 288	Yes n = 30	
Number of men (%)	207 (71.9)	24 (80.0)	0.4624
Age (yr)	47.9±11.4	46.9 (22-73)	0.6453
Number of cirrhosis (%)	90 (31.2)	12 (40.0)	0.4403
Baseline laboratory data			
Total bilirubin (mg/dL)	1.3±1.5	1.1±0.6	0.6895
Albumin (g/dL)	3.9±0.6	3.8±0.6	0.2381
Platelet count (x10⁴/μL)	14.3±5.9	13.2±5.8	0.2624
Number of HBeAg positivity (%)	147 (51.0)	26 (86.7)	0.0004
HBV-DNA[1]	6.6±1.2	7.7±0.7	<0.0001

ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; SD, standard deviation [1]Logarithmic transformed copies/mL

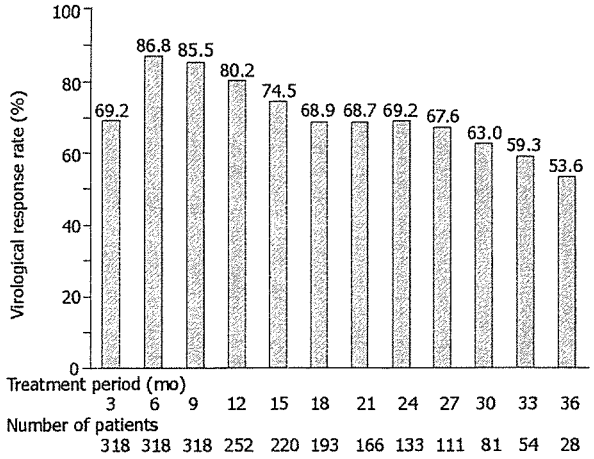

Figure 1 Treatment period and virological response rates to lamivudine treatment of Japanese patients with chronic hepatitis B virus infection.

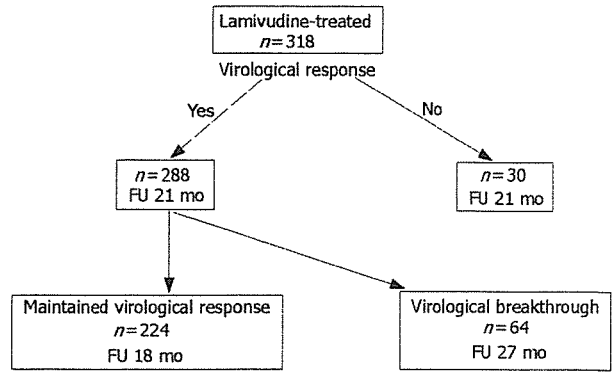

Figure 2 Virological events in all patients during lamivudine treatment period. FU: Follow-up period.

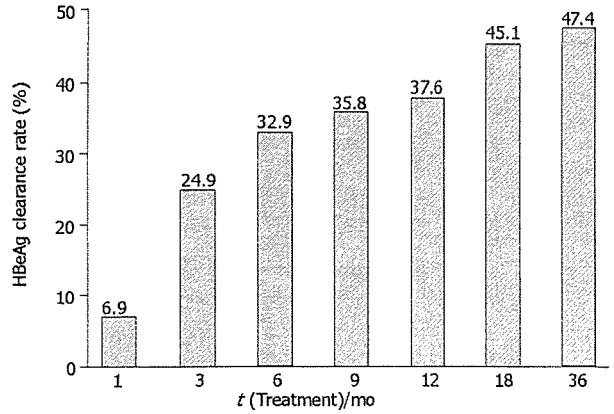

Figure 3 Relationship between treatment period and HBeAg clearance rate during lamivudine treatment of Japanese patients with chronic HBV infection.

(77.8%) had a sustained virological response, and 64 (22.2%) had a virological breakthrough. The median follow-up time was significantly shorter for patients with sustained virological response (18 mo) than those with virological breakthrough (27 mo). The frequencies of pretreatment HBeAg positivity [65.6% (42/64) *vs* 46.8% (105/224); $P=0.0123$] and cirrhosis [43.8% (28/64) *vs* 27.7% (62/224); $P=0.0218$] were significantly higher for patients with virological breakthrough than those without a breakthrough (Figure 2). No significant differences in sex distribution, age, or pretreatment HBV DNA level were observed between these groups.

HBeAg status during lamivudine treatment

Of the 318 patients, 173 (54.4%) were detected to have HBeAg in their sera at baseline. Of the 173 HBeAg-positive patients, 82 (47.4%) had clearance of HBeAg and 91 (52.6%) continued to have HBeAg during treatment. Lamivudine led to HBeAg clearance by 6.9% of the patients at 1 mo, by 24.9% of the patients at 3 mo, by 32.9% at 6 mo, by 35.8% at 9 mo, by 37.6% at 12 mo, by 45.1% at 18 mo, and by 47.4% at 36 mo, suggesting that HBeAg clearance rates increased with the duration of lamivudine treatment (Figure 3). HBeAg clearance always occurred after virological response in all the 82 who cleared HBeAg. No significant differences in sex distribution, age, ALT level, platelet count, presence of cirrhosis, or CTP score were found between the patients with and without HBeAg clearance. Of the 145 patients with HBeAg negative at baseline, no patient reversed to HBeAg positive. We observed that the patients who cleared HBeAg (79/82, 96.3%) and the patients with HBeAg negative at baseline (141/145, 97.2%) had a significantly higher virological response rate than those without HBeAg clearance (68/91, 74.8%, $P=0.0002$, $P<0.0001$, respectively).

ALT breakthrough and hepatitis flare during lamivudine treatment

Of the 274 patients with ALT normalization by lamivudine, 231 (84.3%) had sustained ALT normalization, and 43 (15.7%) had an ALT breakthrough. Of the 43 patients with an ALT breakthrough, 4 (9.3%) had a hepatitis flare: 4 males, 3 with cirrhosis and 1 without cirrhosis, and 4 with HBeAg. However, no patient with hepatic decompensation, who had marked hyperbilirubinemia, or had a liver-related death, was observed in this study. The

Table 3 Forward stepwise logistic regression analysis for all independent factors contributing to virological response

Factors	Odds ratio	95% CI	P
(At baseline)			
HBV DNA less than 6.8[1]	434.7	104.1-2000	< 0.0001
HBeAg negativity	7.142	2.136-238.0	<0.0001
Platelet count more than 100×10^9/L	4.625	1.242-17.22	0.0224
(During treatment)			
Decline of HBV-DNA more than 3.2[1] within 3 mo of the start of treatment	51.13	11.21-233.0	<0.0001

HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; CI, confidence interval of odds ratios [1]Logarithmic transformed copies/mL.

Table 4 Forward stepwise logistic regression analysis for all independent factors contributing to virological breakthrough

Factors	Odds ratio	95% CI	P
(At baseline)			
Cirrhosis	3.527	1.687-7.371	0.0008
HBeAg positivity	2.512	1.265-4.989	0.0085
Platelet counts less than 100×10^9/L	2.386	1.003-5.676	0.0491
(During treatment)			
Decline of HBV-DNA less than 3.9[1] within 6 mo of the start of treatment	2.358	1.246-4.464	0.0084

HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; CI, confidence interval of odds ratios [1]Logarithmic transformed copies/mL.

time of ALT changes always depended on the time of virological change: ALT deterioration after normalization followed an increase in the HBV DNA level in all cases. The frequency of HBeAg positivity at baseline was significantly higher in patients with a breakthrough than those without a breakthrough [72.1% (37/43) *vs* 51.5% (119/231); $P<0.0001$]. No significant differences in sex distribution, age, pretreatment HBV DNA level, presence of cirrhosis or CTP score were observed between these groups.

Relationship between early virological response and virological breakthrough

Among the 288 with virological response, virological breakthrough was seen in 1 (5.3%) of 19 who had virological response at one month, in 42 (20.7%) of 203 at 3 mo, in 14 (27.5%) of 51 at 6 mo, in 4 (33.3%) of 12 at 9 mo, and in 3 (100%) of 3 at ≥15 mo. Cochran-Armitage's trend test revealed that virological breakthrough was significantly more prevalent in patients with delayed virological response ($P<0.0001$) (Figure 4).

Factors contributing to virological response and breakthrough

At baseline, an HBV DNA level less than 6.8 log copies/mL ($P<0.0001$), HBeAg negativity ($P<0.0001$), and platelets count of 100×10^9/L or more ($P=0.0224$) were significantly associated with virological response in the 318 studied patients (Table 3). Of the treatment factors, an early decline of 3.2 or more log copies/mL of HBV DNA at 3 mo after the start of the treatment was significantly associated with the response ($P<0.0001$).

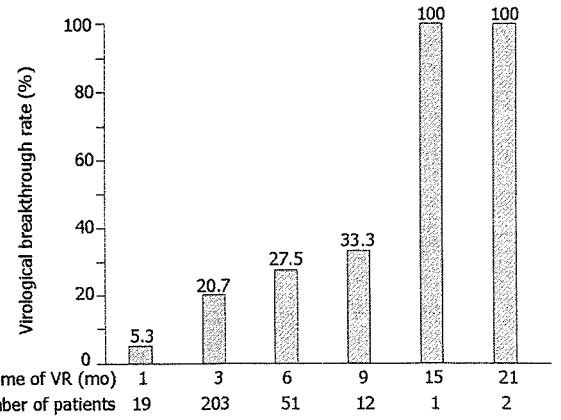

Figure 4 Relationship between time of virological response and virological breakthrough rate during lamivudine treatment of Japanese patients with chronic HBV infection. VR: Virological response.

At baseline, cirrhosis ($P=0.008$), HBeAg positivity ($P=0.0085$), and platelets count less than 100×10^9/L ($P=0.0491$) were significantly associated with a virological breakthrough in the 288 patients with virological response (Table 4). Of the treatment factors, an early decline of 3.8 or less log copies/mL of HBV DNA at 6 mo after the start of the treatment was significantly associated with the breakthrough ($P=0.0084$).

DISCUSSION

To our best knowledge, no such large-scale studies as this of lamivudine have been carried out for Japanese chronic hepatitis B patients. In this retrospective study, good virological and biological efficacy for up to 36 mo of lamivudine treatment was seen in Japanese patients with chronic hepatitis B, with no relation to sex, age, or ALT level at baseline. The effect was sustained for the patients with HBeAg-negative before treatment, absence of cirrhosis, and with an early decline of the HBV DNA level after the start of the treatment. During the treatment, very few patients with a hepatitis flare were seen and none with hepatic decompensation, marked hyperbilirubinemia, or liver-related death were seen in this study. The aims of treatment for chronic hepatitis B are to achieve sustained suppression of HBV replication and remission of inflammation in the liver. The antiviral responses for chronic hepatitis B are categorized as biochemical (ALT normalization), virological (decrease of HBV DNA to less than 5 log copies/mL and loss of HBeAg), and histological, and as on-therapy or sustained off-therapy[14]. Treatment for chronic hepatitis B patients seems to be necessary when the HBV DNA level exceeds 5 log copies/mL, independent of ALT activity[11]. Lamivudine well inhibited HBV DNA replication in Japanese chronic hepatitis B patients.

HBeAg clearance usually predicts long-lasting suppression of HBV, reduced infectivity and an improved clinical prognosis[15]. In this study, 47.4% of patients with HBeAg at baseline had HBeAg eliminated from their sera. Follow-up reports of the multicenter Asian study for Chinese patients showed that HBeAg clearance rates increased with the duration of lamivudine treatment, from 17% to 22% at

12 mo, 27 to 29% at 24 mo, and 33 to 40% at 36 mo[12,16,17]. The results of our study were consistent with those of these non-Japanese patient, although the HBeAg clearance rates within 24 mo were relatively high in our study. Lamivudine was effective in terms of HBeAg clearance in Japanese chronic hepatitis B patients. Patients successfully treated for chronic hepatitis B are less likely to develop cirrhosis, liver failure, and HCC in comparison with those who do not respond to treatment[18]. A randomized controlled trial of lamivudine for chronic hepatitis B patients demonstrated that HCC incidence was reduced by lamivudine antiviral therapy, showing an incidence of 3.9% in lamivudine-treated patients and 7.4% in a placebo control group, with a hazard ratio of 0.49 (95%CI=0.25-0.99)[19]. For chronic hepatitis B patients, antiviral therapy with lamivudine that results in sustained suppression of HBV DNA replication and hepatic necroinflammation may reduce the incidence of HCC.

It has been reported that resistance to lamivudine often develops after 6 mo of treatment[10]. The present study was limited in its value because we detected viruses resistant to lamivudine. However, in our study, the emergence of resistant viruses could be defined by the virological breakthrough (reappearance of serum HBV DNA levels more than 10-fold increase from the minimum). A serious drawback of long-term lamivudine treatment is the development of resistant HBV mutants, i.e., the mutations in a tyrosine-methionine-aspartate-aspartate (YMDD) motif of the HBV polymerase gene, associated with increase in serum HBV DNA and the ALT level[19]. The present study showed that the HBV DNA suppression rates by lamivudine decreased with the duration of treatment, but that a relapse of biochemical response, ALT breakthrough was found only in 15.7% of patients during these treatment periods. Lamivudine treatment withdrawal can cause HBV DNA to revert to pretreatment levels, with the relapse of clinical hepatitis[20]. With the excellent safety and tolerability of lamivudine, continuous therapy is suggested as beneficial[4]. After the start of phylogenetic analyses, based on inter group divergence of 8% or more over the complete HBV nucleotide sequence, seven different genotypes, arbitrarily designed A-G, have been recognized[21,22]. Several reports have shown geographical distribution of the genotypes, with genotypes A and D predominant in Western Europe, B and C in South Asia and the Far East, and F in South America[21-26]. Due to the geographical distribution pattern, HBV genotypes B and C are commonly observed in Japan[24-27]. Moreover, Japan is apparently at a geographical boundary for genotypes B and C, forming a south to north gradient in which genotype C is more frequent in the south of Kyushu, and genotype B is more frequent in the north of Tohoku. Interestingly, however, genotype B is more frequent in Okinawa, the southern-most area of Japan[27]. Our previous epidemiological study of the Japanese HBV genotype distribution showed that 95% of the patients studied had genotype C[24]. Genotype C has been reported to cause more severe liver damage and to have lower rates of HBeAg clearance, which usually indicates cessation of HBV replication and represents a later stage of chronic HBV infection, than genotype B in Japanese patients[24-26]. Accordingly, our results were equivalent in the response to lamivudine to Japanese HBV genotype C patients, although we did not determine the genotyping of our patients.

Another noteworthy finding of our study was that predictive marker of the efficacy to lamivudine and its durability were HBeAg negativity and a low HBV DNA level at baseline. HBV DNA reappears in serum after cessation of lamivudine treatment because HBV replication within the HBV-infected hepatocytes originates primarily from the covalently closed circular DNA (cccDNA) of HBV in the liver. Lamivudine appears to have no effect on the level of cccDNA[28]. Liver injury seems to be particularly severe and rapidly progressive in HBeAg-negative patients, but clinically significant HBV replication persists in them[24]. Most HBeAg-negative chronic hepatitis B patients who are HBV DNA-positive harbor HBV variants with mutations in the precore or core promotor region, which can suppress synthesis of HBeAg[11,26]. The clearance of HBeAg is perhaps a reflection of a loss of the cccDNA pool of HBV in the liver[29]. The great concern of clinicians is that HBeAg negativity and a low HBV DNA level at baseline are significant predictive markers for lamivudine treatment in Japanese patients.

A previous report on Japanese patients showed that the emergence rate of lamivudine-resistant viruses in patients with cirrhosis was higher than those without cirrhosis[28], suggesting that a virological breakthrough appears more frequently in patients with cirrhosis than those without cirrhosis. The present study showed that lamivudine treatment was not so effective or durable in patients with cirrhosis and low platelet counts. Clinicians should always do close monitoring or use other antiviral drugs because hepatitis flare was occasionally severe, especially in patients with cirrhosis. The present study also showed that an early virological response to lamivudine was predictive of both efficacy and durability, but a lack of an early virological response was found to predict a virological breakthrough. A high HBV DNA level reflects a greater pool of virus and a higher rate of virus replication, thereby increasing the likelihood that drug-resistant mutations will be selected. Such an early decrease of viral load after the start of lamivudine might be associated with the lack of viral resistance.

In conclusion, the present study suggests a long-term lamivudine treatment to be safe and to result in the reduction of serum HBV DNA in most Japanese patients with chronic hepatitis B. The efficacy is sustained in patients with HBeAg-negative at baseline, absence of cirrhosis, and a reduction of the HBV DNA level soon after the start of the treatment.

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Original Article

Effect of Probucol on Elderly Hypercholesterolemic Patients in the FAST Study

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Abstract The present study involved a detailed post hoc comparison of the efficacy and safety of lipid-lowering therapy in elderly hypercholesterolemic patients from the Fukuoka Atherosclerosis Trial (FAST). The FAST cohort of 246 hypercholesterolemic patients included 76 patients who were (75 years old. Patients were randomized to receive probucol (500 mg/day) or pravastatin (10 mg/day) therapy, or to a control group (diet alone), and then were followed for 2 years. In patients ≥75 years old, either probucol or pravastatin achieved a significant reduction of carotid intima-media thickness (IMT). In patients <75 years old, lipid-lowering therapy also achieved a significant reduction of IMT. In patients ≥75 years old receiving probucol, the relative risk (95% confidence interval) of all-cause mortality was 0.15 (0.02 to 1.28) and that for major coronary events was 0.12 (0.02 to 1.04). In conclusion, probucol reduced the incidence of cardiovascular disease in elderly hypercholesterolemic patients as well as younger patients.

Key words: elderly hypercholesterolemic patients, probucol, pravastatin, intima-media thickness, carotid atherosclerosis, cardiovascular disease

INTRODUCTION

Atherosclerosis is a common disease in the elderly, and atherosclerotic lesions may cause myocardial infarction or stroke. Measurement of the carotid artery intima-media thickness (IMT) by high-resolution B-mode ultrasonography allows noninvasive detection of early carotid atherosclerosis, and the IMT is also a reliable end-point for trials assessing the effect of interventions on disease progression. Furthermore, ultrasonography can directly quantify early atherosclerotic changes and the response to risk factor modification[1], allowing the use of a smaller patient population to determine the benefits of treatment or accurately assess the presence of early atherosclerosis. The Fukuoka Atherosclerosis Trial (FAST) was the first study to demonstrate the benefit of probucol therapy for patients with hypercholesterolemia and to also reveal an effect of probucol on the incidence of cardiovascular events[2].

A direct relationship between the serum low-density lipoprotein (LDL)-cholesterol level and the risk of coronary heart disease (CHD) has been most clearly demonstrated in studies on middle-aged men. Although a similar relationship has also been observed

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in middle-aged women and in some older populations (≥65 years), the relationship is reported to be weaker in the elderly and it has been less convincingly established for elderly women compared with elderly men[3]. This may be partly because women and elderly patients have been poorly represented in prior clinical trials of cholesterol-lowering therapy. Consequently, the value of screening the lipid profile and performing cholesterol-lowering therapy in these populations is still unclear[4].

The aim of the present study was to perform a detailed post hoc assessment that compared the effect of lipid-lowering therapy on carotid atherosclerosis in younger and older patient subsets (≥75 and <75 years old) from the FAST population.

METHODS

Patient Selection and Study Protocol

The study design and the baseline characteristics of the patients have been described elsewhere[2]. Briefly, between February 1996 and February 2000, men and women aged 30-89 years with hypercholesterolemia who met the following criteria were eligible to participate in the present study. Exclusion criteria included a serum triglyceride level >350 mg/dl, uncontrolled heart failure, recent myocardial infarction (≤6 months), severe or unstable angina pectoris, hypothyroidism/hyperthyroidism or other endocrine diseases, secondary hyperlipidemia, uncontrolled diabetes mellitus, uncontrolled hypertension, heavy alcohol intake, obese patients on weight reduction programs, diseases that might interfere with drug absorption, any severe illness, and treatment with certain drugs (including corticosteroids, androgens, other lipid-lowering agents, or antiacids containing aluminum salts). Hospital visits for monitoring were scheduled after 2 weeks of therapy and then every 4 weeks thereafter. At each visit, a brief physical examination was performed, and the number of tablets was counted to assess compliance. In both groups, lipids, lipoproteins, and other laboratory parameters (to confirm safety) were also measured at each visit. Written informed consent was obtained from each patient, and this trial was approved by the Ethics Committee of Kyushu University Hospital.

The procedure for measurement of carotid IMT and its reproducibility have been described elsewhere[2]. In brief, ultrasonography was done with the patient in the supine position using an Aloka SSD-2000 (Aloka, Tokyo, Japan) with a 7.5 MHz transducer. The IMT of the far wall of both the right and left common carotid arteries was measured at 2, 2.5, and 3 cm proximal to the carotid bifurcation. The IMT was defined as the distance between two echogenic lines separated by a hypoechoic or anechoic space, with the outer line corresponding to the medial-adventitial border and the inner line representing the luminal-intimal border. The mean IMT was calculated as the average value of the measurements obtained at 6 sites (3 per vessel) in the bilateral carotid arteries. Stenosis was defined as plaque (IMT ≥1.10 mm) occupying more than half of the luminal circumference of the artery on a transverse scan.

Laboratory Tests

Blood samples were collected between 8 and 9 am after a 12-hour fast. Serum cholesterol and triglyceride levels were measured by enzymatic methods. Using the calcium heparin method, high-density lipoprotein (HDL) cholesterol was measured in

the supernatant obtained after precipitation of apolipoprotein B-containing lipoproteins by and LDL cholesterol was calculated using Friedewald's formula. Measurements were done on the day of blood collection, or else the blood was stored at -4 (C for no longer than 3 days until assay.

Statistical Analysis

All data were recorded on standard forms and were entered into a database. Results are expressed as percentages or as the mean (SD. The mean values of numerical variables were compared by the Mann-Whitney U test, while categorical variables were compared by the chi-square test, as appropriate.

The endpoint was the effect of each treatment on the incidence of major atherosclerotic events. The relative risk and 95% confidence interval were calculated with the Cox regression model. In all analyses, $P < 0.05$ was considered to indicate statistical significance. All data were analyzed on an intention-to-treat basis.

RESULTS

Baseline Characteristics

The baseline characteristics of the subjects have been summarized elsewhere[2]. Briefly, the mean age of the patients was 66.1 years and 31.3% were men. The average systolic blood pressure and diastolic blood pressure were 130.8 and 77.1 mm Hg, respectively. Of the 246 patients, 59.3% were recent or former smokers, 42.5% had a history of hypertension, and 22.9% had diabetes mellitus. Baseline serum total cholesterol and LDL-cholesterol levels were 253.0 mg/dL and 166.1 mg/dL, respectively, while the HDL-cholesterol level was 57.0 mg/dL and the serum triglyceride level was

Table 1 Baseline characteristics (including lipids) for the two subgroups of interest (patients ≥75 years old and patients <75 years old)

		Probucol			Pravastatin			Diet alone		
		Age≥75 (n=27)	Age<75 (n=55)	chi-square test	Age≥75 (n=27)	Age<75 (n=55)	chi-square test	Age≥75 (n=22)	Age<75 (n=59)	chi-square test
		No.(%)	No.(%)		No.(%)	No.(%)		No.(%)	No.(%)	
Sex	M	4(14.8)	21(32.0)	0.0568	1(3.6)	21(32.0)	0.0018	6(27.3)	24(40.7)	0.3939
	F	23(85.2)	34(61.8)		27(96.4)	34(61.8)		16(72.7)	35(59.3)	
Smoking	+	12(44.4)	36(65.5)	0.1149	13(46.4)	31(56.4)	0.5321	15(68.2)	39(66.1)	1.0000
	−	15(55.6)	19(34.5)		15(53.6)	24(43.6)		7(31.8)	20(33.9)	
CVD	+	17(63.0)	18(32.7)	0.0181	19(67.9)	15(27.3)	0.0009	14(63.6)	20(33.9)	0.0308
	−	10(37.0)	37(67.3)		9(32.1)	40(72.7)		8(36.4)	39(66.1)	
IHD	+	5(18.5)	4(7.3)	0.2480	8(28.6)	7(12.7)	0.1410	7(31.8)	4(6.8)	0.0104
	−	22(81.5)	51(92.7)		20(71.4)	48(87.3)		15(68.2)	55(93.2)	
HT	+	12(44.4)	25(45.5)	1.0000	16(57.1)	23(41.8)	0.2757	9(40.9)	17(28.8)	0.4415
	−	15(55.6)	30(54.5)		12(42.9)	32(58.2)		13(59.1)	42(71.2)	
DM	+	3(11.1)	12(21.8)	0.3818	5(17.9)	13(23.6)	0.7472	4(18.2)	19(32.2)	0.3331
	−	24(88.9)	43(78.2)		23(82.1)	42(76.4)		18(81.8)	40(67.8)	

	Probucol				p-value Mann Whitney's U-test	Pravastatin				p-value Mann Whitney's U-test	Diet alone				p-value Mann Whitney's U-test
	Age≥75(n=27)		Age<75(n=55)			Age≥75(n=27)		Age<75(n=55)			Age≥75(n=22)		Age<75 (n=59)		
	No.	Mean S.D.	No.	Mean S.D.		No.	Mean S.D.	No.	Mean S.D.		No.	Mean S.D.	No.	Mean S.D.	
sBP	27	73.3±23.1	55	133.3±22.6	0.8434	28	130.2±24.4	55	128.7±22.0	0.5369	22	141.5±24.5	59	127.0±17.0	0.0077
dBP	27	21.6±11.5	55	80.0±13.1	0.0526	28	71.5±10.2	55	77.7±11.3	0.0187	22	78.7±8.2	59	77.4±10.2	0.2531
BMI	27	21.6± 3.3	55	23.9± 4.5	0.0109	28	21.8± 5.7	55	24.4±3.4	0.0118	22	22.3±2.1	59	23.1± 2.9	0.1723
IMT	27	1.5± 0.8	55	1.3± 0.5	0.7556	28	1.4± 0.9	55	1.2±0.4	0.6748	22	1.3±0.5	59	1.3± 0.5	0.8943
TC	27	257.9±25.5	55	249.7±25.5	0.0962	28	258.0±23.5	55	248.2±25.1	0.0349	22	256.0±23.3	59	254.9±30.3	0.3895
LDL-C	27	171.3±31.1	55	163.5±24.7	0.2734	28	161.5±29.2	55	160.2±33.0	0.4325	22	175.2±27.1	59	170.2±36.3	0.4509
HDL-C	27	61.1±19.9	55	56.2±18.7	0.2864	28	62.2±14.3	55	53.9±15.6	0.0218	22	55.1±10.0	59	57.0±16.5	0.8400
TG	27	127.9±56.0	55	150.2±72.3	0.2119	28	171.8±75.0	55	167.1±89.8	0.4880	22	128.5±44.8	59	138.5±63.7	0.6479

CVD: cerevral vascular disease
IHD: ischemic heart disease
HT: hypertension
DM: diabetes mellitus
sBP: systolic blood pressure
dBP: diastolic blood pressure
BMI: body mass index
IMT: intima-media thickness
TC: total cholesterol
LDL: LDL-cholesterol
HDL: HDL-cholesterol
TG: triglyceride

149.2 mg/dL. The mean IMT was 1.308 mm. There were no statistically significant differences in any of these baseline characteristics among the three treatment groups (probucol, pravastatin, and diet alone).

Baseline characteristics (including lipids) for the two subgroups of interest (patients ≥75 years old and patients <75 years old) are shown in the Table 1. In general, the three treatment groups were well matched for age and sex at baseline. The elderly subgroup (≥75 years old) included a higher proportion of women, and more patients had cerebrovascular disease compared with the younger subgroup (<75 years old) ($p<0.01$ for probucol, $p<0.01$ for pravastatin, and $p<0.01$ for diet alone; chi-square test). The potential importance of chance differences in baseline characteristics between any of the four subpopulations receiving either of the two active treatments was evaluated by assessing the relationship of all listed baseline variables to total mortality or major coronary events.

Drug Treatment and Serum Lipids

The percent changes of serum lipids after 2 years of treatment are displayed in Fig. 1. Mean between-group differences (intention-

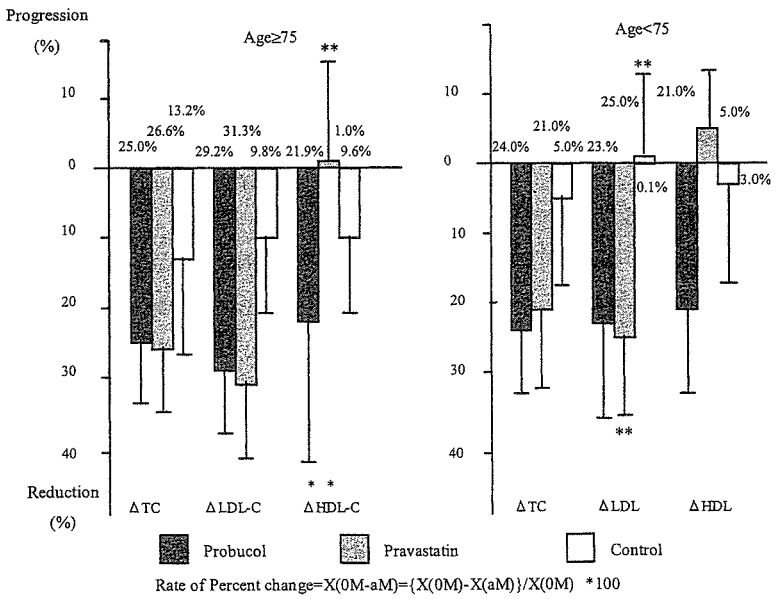

Fig. 1 Percent changes of serum lipids after 2 years. Among patients ≥75 years old, there was a significant decrease of serum total cholesterol in each of the three subgroups (by 25.0%, 26.6%, and 13.2% compared with baseline, respectively). There was also a significant decrease of serum LDL-cholesterol by 29.2%, 31.3%, and 9.8%, respectively, while the HDL-cholesterol levels of the probucol and control groups were significantly lower after 2 years. Patients <75 years old from the probucol and pravastatin groups showed a significant decrease of serum total and LDL-cholesterol levels (by 23.6% and 21.0% or 23.3% and 25.5% compared with baseline, respectively). In the probucol group, HDL-cholesterol was significantly reduced after 2 years (21.9%, $p<0.01$).

to-treat) of the percent change from baseline over the full duration of the trial are shown for total cholesterol, LDL cholesterol, and HDL cholesterol.

Patients ≥ 75 Years Old

After 2 years of treatment, there was a decrease of serum total cholesterol in each of the three groups, which showed a significant reduction of 25.0%, 26.6%, and 13.2% compared with baseline, respectively. After 2 years, there was also a significant decrease of serum LDL-cholesterol in the three groups, with the reduction being 29.2%, 31.3%, 9.8%, respectively. The serum HDL-cholesterol level of the pravastatin group was increased by 1.0% after 2 years, but this change was not significant. On the other hand, the HDL-cholesterol level showed a significant decrease in the probucol and control groups by 21.9% and 9.6%, respectively (Mann-Whitney U test). Triglyceride levels showed no significant changes throughout the study.

Patients < 75 Years Old

After 2 years of treatment, there was a significant decrease of serum total cholesterol and LDL-cholesterol levels in the probucol and pravastatin groups, with a reduction of 23.6% and 21.0% versus 23.3% and 25.5% compared with baseline, respectively. In the control group, total cholesterol and LDL-cholesterol levels were also lower at the end of the study, but the changes were not significant. The HDL-cholesterol level of the probucol group was significantly reduced after 2 years (21.9%, $p<0.01$). In the pravastatin group and the control group, however, HDL-cholesterol showed no significant changes throughout the study. Triglyceride levels also showed no significant changes throughout the study in any of the groups.

Intima-Media Thickness

The percent change of carotid IMT after 2 years is shown in Fig. 2. The decrease of IMT in patients ≥75 years old from the

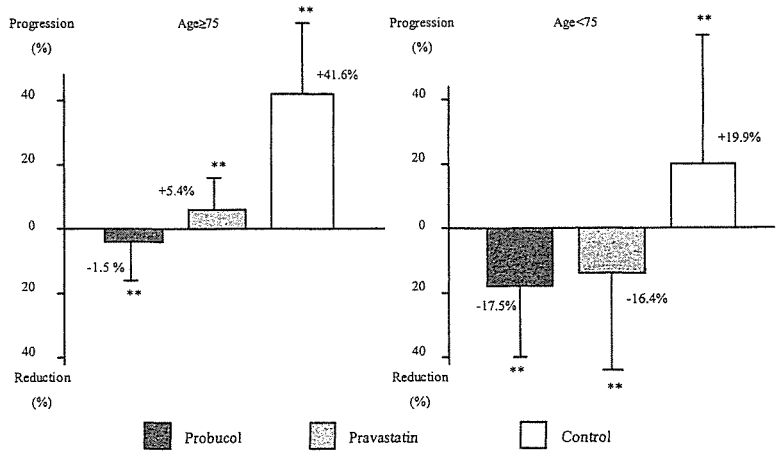

Fig. 2 Percent changes of carotid IMT after 2 years. Among patients ≥75 years old from the probucol and pravastatin groups, IMT showed a significantly greater decrease compared with that in patients <75 years old (both $p<0.01$). In the control group, IMT increased significantly by 19.9% ($p<0.05$). The change of IMT was significantly different in the treated groups compared with the control group (both $p<0.001$).

probucol and pravastatin groups was significant compared with that in patients <75 years old (both $p<0.01$; Mann-Whitney U test). In the control group, however, IMT showed a significant increase of 19.9% after 2 years ($p<0.05$; Mann-Whitney U test). The changes of IMT in the probucol and pravastatin groups were significantly different compared with that in the control group (both $p<0.001$; Mann-Whitney U test), while there was no significant difference in the change of IMT between the probucol and pravastatin groups at 24 weeks after the completion of treatment. There was no significant increase of IMT in the probucol group after 2 years of treatment.

Total and CHD Mortality

Among the 82 patients in the probucol group, two suffered a major cardiovascular event (2 deaths from coronary heart disease). Major events occurred in 4 of the 83 patients from the pravastatin group (3 deaths from coronary heart disease and 1 nonfatal myocardial infarction) and 11 of the 81 patients from the control group (8 deaths from coronary heart disease and 3 nonfatal myocardial infarctions). Of the 16 deaths that occurred during this study, two were in the probucol group, 5 were in the pravastatin group, and 9 were in the control group. Among these 16 patients, 13 deaths were from cardiovascular causes, while the others were due to gastrointestinal bleeding and infection. Total mortality and CHD mortality in the patients ≥75 years old are shown in Table 2.

Total cardiovascular events were significantly reduced in patients ≥75 years old from the probucol group compared with the control group (relative risk: 0.12; $p<0.05$). The reduction of relative risk was slightly greater than that observed for patients <75 years old (relative risk: 0.20; p=N.S.), but there were overlapping 95% confidence intervals. The relative risk of total death was similarly reduced by probucol in both age groups (86% reduction for patients ≥75 years old), and this decrease was statistically significant. Although the relative risk of total death was also reduced by pravastatin in both age groups (43% reduction for patients ≥75 years old), the change was not significant. The total cardiovascular event rate and total death rate over the duration of the study were more than three times higher in control group patients ≥75 years old (27.3% and 22.7%, respectively) compared with patients <75 years old (8.5% and 6.8%, respectively). Conse-

Table 2 Effect of probucol and pravastatin on clinical events in hypercholesteromoic patients

		Patients, n (%)													
		Probucol n=82		Pravastatin n=83		Control n=81		Probucol				Pravastatin			
								Hazards ratio	95%C.I.		p	Hazards ratio	95%C.I.		p
Age≥75		n=72		n=28		n=22									
	All cardiovascular events	1	(3.7)	4	(14.3)	6	(27.3)	0.1247	0.0150	1.0358	0.0184	0.476	0.1343	1.6875	0.2439
	Fatal MI	1	(3.7)	3	(10.7)	5	(22.7)	0.1509	0.0176	1.2923	0.0416	0.4317	0.1031	1.8072	0.2403
	Non-fatal MI	0	(0.0)	1	(3.6)	1	(4.5)					0.6899	0.0431	11.0317	0.7936
	PTCA/CABG	0	(0.0)	1	(3.6)	0	(0.0)								
	All cerebrovascular events	0	(0.0)	0	(0.0)	0	(0.0)								
	All other events	0	(0.0)	1	(3.6)	0	(0.0)								
	All deaths	1	(3.7)	4	(14.3)	5	(22.7)	0.1498	0.0175	1.2828	0.0407	0.5713	0.1533	2.1287	0.4023
Age<75		n=55		n=55		n=59									
	All cardiovascular events	1	(1.8)	0	(0.0)	5	(8.5)	0.2080	0.0243	1.7804	0.0968				
	Fatal MI	1	(1.8)	0	(0.0)	3	(5.1)	0.3430	0.0357	3.2978	0.3197				
	Non-fatal MI	0	(0.0)	0	(0.0)	2	(3.4)								
	PTCA/CABG	0	(0.0)	0	(0.0)	0	(0.0)								
	All cerebrovascular events	0	(0.0)	0	(0.0)	0	(0.0)								
	All other events	0	(0.0)	1	(1.8)	1	(1.7)					1.3237	0.0823	21.2987	0.8434
	All deaths	1	(1.8)	1	(1.8)	4	(6.8)	0.2557	0.0286	2.2879	0.1726	0.2990	0.0334	2.6794	0.2322

quently, the absolute risk reduction for patients ≥75 years old was more than three times that for patients <75 years old in the case of both total cardiovascular events and total deaths.

DISCUSSION

FAST was the first clinical trial to clearly demonstrate the benefit of probucol for elderly hypercholesterolemic patients and to also demonstrate an effect of probucol on the incidence of cardiovascular events. FAST showed that probucol therapy could achieve a significant reduction in the risk of major coronary events in patients ≥75 or < 75 years old, as well as significant improvement of all the tertiary CHD and atherosclerosis-related study end-points that were positive in the entire FAST cohort. The magnitude of the observed risk reduction in these subgroups was very similar to that reported for the entire study cohort and for other clinically relevant subgroups that have been analyzed. Although FAST was not specifically designed to assess changes of mortality in elderly subjects, high event rates combined with the substantial percentage of patients in this subgroup allowed us to detect a significant reduction of both all-cause mortality and CHD mortality. Safety and tolerability showed no important differences between the two age groups and were largely consistent with the findings for the entire study cohort[2].

In the subjects ≥75 versus <75 years old, LDL cholesterol showed similar changes (26% vs. 22%). This finding is consistent with other data suggesting that the cholesterol-lowering effect of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors is enhanced as patients become older[5]. Baseline total cholesterol, LDL-cholesterol, and HDL-cholesterol levels showed no significant relationship with the response to treatment (reduction in relative risk) in any of the subpopulations examined (data not shown), as was also the case for the entire study cohort[2]. The reduction of LDL cholesterol was more significant in the pravastatin group than in the probucol or control groups. Although the control group showed a significant reduction of LDL cholesterol with diet alone, an increase of carotid IMT still occurred, unlike the outcome in the active treatment groups. After 2 years of therapy, there was a significant decrease of serum LDL-cholesterol in all three groups compared with baseline. It was interesting that probucol had an antiatherogenic effect and caused a reduction of CHD events in patients ≥75 years old.

Lipid peroxidation of LDL has been demonstrated to be an important risk factor for the development of atherosclerosis[6][7]. There are several possible reasons, including the increased susceptibility of LDL to oxidation with aging[8], which can be partly explained by modification of its fatty acid composition and a decrease of the antioxidant (vitamin E) content[8]. Recently, Napoli et al. reported that resistance of LDL to peroxidative modification was lower in elderly men than in young men[9]. Furthermore, age was correlated with the extent of lipid peroxidation, supporting the hypothesis that LDL contributes to the increment of plasma lipid peroxides with aging[10][11]. Since oxidation of LDL is considered to be a key event in atherogenesis, it could be an additional reason why atherosclerosis is related to aging.

FAST showed that probucol therapy could delay the increase of IMT independently of its LDL or HDL cholesterol-lowering effect, and a reduction of IMT occurred

earlier with probucol than with pravastatin[2]. In the present study, patients ≥75 years old showed a significantly smaller change of IMT after probucol therapy compared with patients < 75 years old irrespective of the cholesterol-lowering effect. However, it was clearly demonstrated that probucol could reduce the risk of all-cause mortality and major coronary events in CHD patients ≥75 years old. The above findings suggest that there may be another mechanism involved in the effect of probucol. Other investigators have shown that suppression of atherogenesis by probucol is independent of its cholesterol-lowering action and is presumably due to an antioxidant effect on lipids[12)13)]. Because mortality and CHD events increase with age[14)], the absolute reduction of the death rate and event rate was substantially greater for patients ≥75 years old compared with those <75 years old. The relationship between serum cholesterol and the development of CHD has been observed in various epidemiological studies, but is reported to be weaker in elderly persons compared with middle-aged subjects[15)~19)], so the above findings may be unexpected. However, limited data are available about the predictive value of cholesterol in elderly patients with established CHD. Taken together with the results of the present study, the above findings may indicate the importance of ancillary effects of probucol other than cholesterol lowering for reducing the incidence of cardiovascular events. In fact, our data suggest that probucol may have multiple actions, but further studies are needed to investigate the relative contribution of each effect of this drug.

A difference between the effect of probucol and pravastatin on the IMT was not demonstrated by the present study, perhaps because the sample size was small. A large-scale investigation would be necessary to determine whether probucol and pravastatin therapy have a different influence on the IMT. Lack of a placebo control group was another limitation of our study. However, the use of quantitative B-mode ultrasound allowed us to obtain unbiased data. Although FAST was not specifically designed to assess the influence of lipid-lowering therapy on mortality in the elderly, high event rates combined with the substantial percentage of elderly patients in the study population provided the power to demonstrate a significant reduction of both all-cause mortality and CHD mortality among elderly patients receiving probucol. Safety and tolerability showed no important differences related to age or sex, and were generally consistent with the results for the entire study cohort[2)].

In conclusion, the present findings suggest that hypercholesterolemia in the elderly is a morbid state requiring treatment and that probucol is a useful drug for reducing the incidence of cardiovascular disease in hypercholesterolemic elderly persons.

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(和文抄録)

高齢者の高コレステロール血症に対するProbucolの効果

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【目的】高齢者（75歳以上）の高コレステロール血症患者に対して，積極的な脂質低下療法が頚動脈硬化の進展抑制および主要冠動脈イベントリスク低下が認められるか否かについて検討した．
【方法】FASTの対象患者（246例）のうち，75歳以上（76例）と75歳未満（168例）について，脂質低下療法（Probucol Pravastain）および食事療法により，その有効性について頚動脈エコーを用いて評価した．総頚動脈の内膜中膜複合体厚(IMT)を測定し，左右6点のIMTの平均値をIMT値とした．1次エンドポイントは2年間のIMT値の変化率とし，2次エンドポイントは主要冠動脈イベントとした．
【結果】Probucol群及びPravastain群では，年齢に関係なく，高齢者においても動脈硬化の進展抑制を認めた．Probucol群における高齢者のControl群に対する各臨床イベントの相対リスク（95%信頼期間）は総死亡が0.15（0.02-1.28），総冠動脈イベント0.12(0.02-1.04)と有意な進展を認めた．一方，Pravastain群との間では，各臨床イベントの相対リスクに有意差は認められなかった．Probucol群とPravastain群との間では，各臨床イベントの相対リスクに有意差は認められなかった．
【結論】75歳以上の高齢者に対してもProbucolは，頚動脈硬化の進展抑制効果が認められ，さらに主要冠動脈イベントの相対リスクの低下作用を認められる可能性が示唆された．