A Randomized-Controlled Study investigating Viral Suppression and Serological Response following PreS1/PreS2/S Vaccine Therapy Combined with Lamivudine in HBeAg-positive Chronic Hepatitis B Patients

Running title: Triple Vaccine Combined with Lamivudine in Chronic Hepatitis B

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Abstract The aim of the current study was to evaluate viral suppression following combined S/preS1/preS2 vaccine and lamivudine treatment in patients with chronic hepatitis B. We established a randomized, controlled clinical trial to compare the response between three different treatment groups including vaccine monotherapy, lamivudine monotherapy and combination treatment. Viral response was evaluated via HBV-DNA suppression using different levels of classification. Seroconversion was evaluated via HBeAg loss, HBeAg sero-conversion and HBsAg loss and anti-HBs response. We found that the group receiving combination treatment demonstrated a significant increase in viral suppression compared to the lamivudine or vaccine monotherapy groups, although the HBeAg sero-conversion was not different. This enhanced suppression effect in the combination group was reversed after discontinuation of vaccine treatment, suggesting that booster doses are required for a sustained viral response. Anti-HBs were detected in 55/120 vaccine recipients but only three patients demonstrated HBsAg loss, indicating that the vaccine-induced anti-HBs was unable to completely neutralize HBsAg in the serum. At the study endpoint, anti-HBs responders showed significantly increased HBeAg sero-conversion, suppression of HBV-DNA levels, and a lower median HBV-DNA reduction compared to the anti-HBs non-responders. Our results suggest that the combined treatment of vaccine and lamivudine was significantly more effective than lamivudin**e** monotherapy in the short term, and was especially successful for viral suppression and enhanced anti-HBs antibody response.

Introduction

Chronic hepatitis B virus infection (CHB) is well established as a major health problem world-wide. Each year, more than 500,000 deaths are reported from an estimated 350 million individual sufferers, due to complications in CHB-related chronic liver disease (19, 22, 29). CHB is characterized by periodic activation of the host immune system against infected hepatocytes. This activation is often unable to eradicate the virus, and usually progresses to liver fibrosis, cirrhosis and even hepatocellular carcinoma (3, 31). Therefore, antiviral or immune therapy is generally administered in the aim of suppressing viral replication, eradicating the virus, and normalizing alanine aminotransferase (ALT) levels to prevent hepatocyte necrosis and cirrhosis. This is especially important in CHB patients exhibiting high viremia and high ALT (usually higher than two times the upper limit of normal > 2 ULN (Upper normal limit)) (8, 27).

Interferon (IFN) and nucleoside analogues are the main therapies currently used in the treatment of CHB (27). However, treatment with IFN is costly, has limited positive effects and numerous important side-effects (27). Antiviral nucleoside analogues have rapid virological suppression effects but are often unable to sustain the response when therapy is discontinued. In addition, the selection of hepatitis B virus (HBV) resistant or complex mutation strains after long-term therapy with these agents have also been reported (25, 33), and lead to virological and hepatitis breakthrough (23, 39). Furthermore, adefovir, the antiviral nucleoside analogue used to treat chronic hepatitis B, has also been associated with nephrotoxicity following long-term therapy

(11). These issues have promoted the development of novel therapeutic approaches in the treatment of CHB patients including immunomodulatory drugs, adoptive transfer of immunity and therapeutic vaccination (16).

Differences in antigenic structure have been investigated in experimental vaccine therapy studies, in order to enhance both the humoral and cytotoxic T-cell response during the eradication of infected liver cells. The third generation recombinant triple hepatitis B vaccine, which contains pre-S1, pre-S2 and S antigenic components derived from mammalian cells, has shown significantly enhanced immunogenicity when compared to conventional yeast-derived vaccines, and has been used in immunotherapy (43, 45). It has been reported that therapeutic vaccination with GenHevacB® (Aventis Pasteur, France) or with Recombivax® (Merck Sharp Dohme-Chibret, France, containing S and pre-S2 antigen) significantly reduces HBV-DNA levels and leads to a more rapid HBeAg sero-conversion in chronic adult HBV carriers (37), a response that likely occurs via induction of CD4+ T lymphocyte response to envelope antigens (6). In addition, vaccine monotherapy studies using various antigenic structures, have also been investigated in different study populations, and have shown a range of effects in HBeAg sero-conversion and viral inhibition. However, only a modest HBsAg sero-conversion was observed in CHB patients (6, 36, 42).

Studies investigating combined vaccine and lamivudine (LAM) treatment have also been reported (12, 17, 35, 41). The effects of combination treatment when compared to LAM

monotherapy appears to be quite variable, a result that is thought to be due to variations in the timing of the vaccination, the type and dose of vaccine, variations in the duration of vaccine intervention, and even in differences in the selection criteria of the study population. Therefore, we performed a randomized, controlled trial to evaluate the effects of combination treatment with a double dose of triple hepatitis B vaccine (Sci-B-vac, BTCG USA, SciGen, Ltd.) and LAM in CHB patients during the active disease state. The effects of treatment intervention were based on viral suppression and sero-conversion evaluated at different time points. The association between viral response and serological anti-HBs response were also analyzed. At the commencement of our study, LAM was considered the standard initial treatment for CHB patients (28, 38), and continues to be one of the current treatments of choice for naïve CHB patients in Vietnam (40).

MATERIALS AND METHODS

Patient selection. The prospective, open-label study was carried out in the outpatient department of the Hospital for Tropical Diseases of Ho Chi Minh City (Vietnam). 180 patients exhibiting CHB were enrolled in the study from 2003 to 2006, and met the following entry criteria: (i) were adult male/female aged between 16–70 years old; (ii) demonstrated positive HBsAg on two separate samples assayed during the 6 months prior to the onset of the study (iii) demonstrated positive HBeAg, no detectable anti-HBs, anti-HBc IgM or anti-HBe antibodies; and (iv) exhibited elevated serum ALT levels that were higher than 2 (>2 ULN), but were less than 10

times the upper limit of the normal (<10 ULN) range for at least 2 consecutive tests during 3 months before enrolment in the study. Patients positive for HCV, HDV or HIV, and those found to have a history of other liver diseases including autoimmune diseases, alcoholic liver disease and metabolic diseases, or kidney disease were excluded from the current study.

The study protocol was approved in advance by the Ethical and Scientific Committee of the Hospital for Tropical Diseases of Ho Chi Minh City, and was performed according to the Declaration of Helsinki. All patients signed an informed consent prior to enrolment into this study. Clinical examination and laboratory tests including serum ALT, blood creatinine and complete blood counts, were performed every month during the first 9 months and every 3 months afterward. HBsAg, anti-HBs, HBeAg, anti-HBe and HBV-DNA levels were measured every 3 months, and until 18 months after inclusion into the study.

Study design. Patients were randomly assigned at a 1:1:1 ratio to one of the following groups: (1) vaccine monotherapy (VAC group); (2) combination LAM and vaccine therapy (V+L group) and (3) LAM monotherapy (LAM group) (Fig. 1). The patients in the V+L and LAM groups were treated orally with 100 mg LAM (STADA) once daily. LAM treatment was discontinued in patients who exhibited HBeAg loss for 6 months. Patients in the VAC and V+L groups received a 20 µg intramuscular injection (doubled adult dose of commercial triple vaccine Sci-B-Vac, BTGC USA, SciGen Ltd) into the deltoid muscle, each month for 8 consecutive months.

Evaluation. Following the initiation of the study (baseline), patients were subjected to routine laboratory tests (ALT) and observed for side-effects. Efficacy analyses were calculated for all patients who received at least one dose of drug or vaccine, in accordance to the intention-to-treat principle (32). Patients missing values at any time point were classified as exhibiting no response.

A reduction of more than 1 or 2 log copies/ml of HBV-DNA from baseline after the first 3 months was considered to be a rapid virologic response, termed the primary response. A biochemical response was defined as a normalization of serum ALT levels during the study period (<1.5 ULN in two consecutive tests). HBeAg loss was indicated by a disappearance of HBeAg, while HBeAg sero-conversion was defined by a loss of HBeAg in conjunction with the appearance of anti-HBe antibody. Anti-HBs antibody levels of ≥10 mIU⁄ml were considered to represent an anti-HBs response. HBsAg sero-conversion was indicated by a loss of HBsAg, in conjunction with the appearance of anti-HBs antibody.

To analyze for a sustained response (off-vaccine treatment response), we classified the response after 18 months compared with that after 9 months into 4 categories: sustained response (sustained HBeAg loss or HBV-DNA suppression from 9 to 18 months), additional response (no response after 9 months, but response after 18 months), non-response (no response observed during the study), and HBeAg reversion (HBeAg loss after 9 months, but positive after 18 months) or HBV-DNA breakthrough (HBV-DNA elevated to > 4 log copies/ml on cases that HBV-DNA suppressed < 4 log copies/ml after 9 months).

Serum HBV-DNA levels were quantified using a commercial quantitative real-time polymerase chain reaction (Q-PCR) assay using Taqman probes with a low sensitivity detection limit (≥500 copies/ml). HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HCV, anti-HDV and anti-HIV antibodies were detected using the microparticle enzyme immunoassay (MEIA), ABBOTT [Max-Planck-Ring 2 65205, Wiesbaden, Germany].

Statistical analysis. The data for HBV-DNA in each group before and after treatment is presented as median values and was compared using the median test. The rate of response in the groups was reported as a percentage and was analyzed using the chi-square test or Fisher's Exact test for the subgroups with a small number of responses. P-values less than 0.05 were considered statistically significant. Missing values were not included when the mean and median values were calculated and compared, and the number of patients per group was also adjusted before the analysis. All statistical analysis was performed using the SPSS 10.0 program.

RESULTS

Baseline characteristics. At baseline, we observed similar biochemical and virological characteristics in the three treatment groups with respect to both age and sex ($p>0.05$, Table 1). Although the median value of the combination group (6.1 log copies/ml) was slightly lower than those of the other groups (6.8 and 6.5 log copies/ml in the VAC and LAM monotherapy group,

respectively), these differences were not statistically significant when assessed using the median test (p=0,058). The majority of the study population was found to be male (63.3%), and 15% had been treated previously with LAM for hepatitis B. These subjects had ceased LAM treatment more than 6 months prior to the onset of the study. No patients had received prior interferon treatment. Of the 180 subjects initially enrolled, only 14 patients discontinued the study prior to completion. 3 patients exited the vaccine group after 12 months, 2 from the combination group after 14 months, and 9 from the LAM group after at least 14 months of treatment.

We did not observe any serious side-effects, intense biochemical flare or hepatic decompensation due to immune induction in any patient during the treatment and follow-up periods.

HBeAg loss and HBeAg sero-conversion. Our results showed that HBeAg sero-conversion in the 3 groups was approximately 7-20%, 18-27% and 21-33% after 3, 12 and 18 months of treatment, respectively. The rate of HBeAg sero-conversion gradually increased over the study period in the 2 vaccine groups, but remained unchanged in the LAM monotherapy group (Table 2). We did not observe any significant differences between the 3 treatment groups in terms of HBeAg loss and HBeAg sero-conversion over the course of the study.

HBV-DNA reduction. At the end of the third treatment month, the rate of HBV-DNA reduction (>1 log copy/ml) from baseline was found to be significantly higher in the V+L and LAM groups (55% and 28.3%) compared to the VAC monotherapy group (8.3%, p<0.001). The

median reduction in HBV-DNA after 3 months was also significantly higher in the V+L group (-2.6 log copy/ml) and in the LAM group (-1.9 log copy/ml) when compared to the VAC monotherapy group (0.0 log copy/ml), suggesting an early virological response related to LAM treatment (Table 2, $p<0.001$). Furthermore, virological response with HBV-DNA reduction of greater than 2 log copies/ml from baseline (53.3% vs. 31.7%, Fig. 2, $p=0.016$. RR=1.68) and the rate of HBV-DNA suppression (<4 log copies/ml, 55% vs. 28.3%, Table 2, $p<0.001$) were also significantly higher in the V+L group compared to the LAM group. The viral suppression (HBV-DNA levels <4 log copies/ml) from months 3 to 12 were also significantly higher in the combination group compared to the LAM group ($p<0.05$, Table 2). However, the enhanced suppression effects in the combination group compared to LAM monotherapy was lost during follow-up (after 12-18 months).

Biochemical response. In the early stages of the current study (3-12 months), a significantly increased number of patients receiving treatment with LAM, both in the V+L and LAM groups, exhibited normal ALT when compared to those receiving treatment with VAC ($p<0.05$, Table 3). However, at the end of the study (18 months), the rate of biochemical response was similar for all 3 treatment groups.

HBsAg loss, appearance of anti-HBs and HBsAg sero-conversion. Four patients experienced HBsAg loss during the follow-up period; 1 patient in the vaccine monotherapy group demonstrated HBsAg loss after 12 months, 2 combination group patients after 14 and 18 months,

and 1 LAM monotherapy patient after 18 months. Anti-HBs response was detected in 50 of the 120 vaccine recipients (27.8%), but not detected in patients of the LAM group. HBsAg sero-conversion, that is HBsAg loss with anti-HBs detection, was observed in 2 patients in the VAC monotherapy group (1 after 15 months and 1 after 18 months). However, anti-HBs were not detected in 2 patients of the V+L group and the LAM group that experienced HBsAg loss.

The rate of anti-HBs responses (≥10 mIU/ml) in both the VAC and V+L groups increased in a time-dependent manner during the study period (Table 4). Additionally, the proportion of anti-HBs responders was statistically higher in the V+L therapy group compared with the VAC monotherapy group. Furthermore, 49% of patients with anti-HBs detection (27/55 cases) also exhibited high levels of anti-HBs (between 100 and 1000 mIU/ml) after 12 months.

At the end point of this study (month 18), anti-HBs responders demonstrated a significantly higher proportion of HBeAg sero-conversion (40% versus 21.4%, $p=0.027$), a higher rate of HBV-DNA suppression (<4 log copies/ml, $p<0.001$), and a lower median HBV-DNA reduction ($p=0.027$) than the anti-HBs non-responders (Table 5).

The off-treatment response. The off-treatment serological and virological responses among the 3 treatment groups are presented in Tables 6 and 7. The results revealed that HBeAg reversion was observed following the LAM monotherapy and the V+L combination therapy. In addition, the V+L combination group demonstrated a lower rate of virological non-response (28.3% vs. 48.3%) and a higher rate of sustained or additional HBV-DNA response (51.7% vs. 43.3%) when

compared to the patients in the LAM monotherapy group. However, the combination group also demonstrated a higher rate of viral breakthrough than the LAM monotherapy group (20.0% vs. 8.3%, Table 7, p=0.04).

DISCUSSION

The practical aim of CHB therapy is to suppress HBV replication, normalize ALT levels and induce HBeAg sero-conversion in order to prevent disease progression to cirrhosis and hepatocellular carcinoma. Treatment options for CHB patients are limited to two approved therapeutic approaches including treatment with interferon and nucleoside analogues. Our novel approach using therapeutic vaccination combined with LAM was thought to be effective in generating potential synergistic or additive effects. LAM is known to directly reduce viral load, while cytokines released following vaccine therapy may enhance the cellular immune response to suppress viral replication or to clear the virus. The limited combination therapy studies in CHB patients using HBV vaccine and LAM conducted to date have shown varied results for HBeAg sero-conversion and viral suppression (12, 17, 41). Very few reports have noted any anti-HBs response after vaccine therapy or their effects on viral clearance.

In the natural course of HBV infection, HBeAg sero-conversion has been recognized as an important event. Early HBeAg sero-conversion suggests a better clinical outcome, while an absent or late HBeAg sero-conversion after multiple hepatitis flares enhances progression to cirrhosis (5,

15). In HBeAg-positive CHB patients, the current aims of treatment are to obtain a biochemical response, HBeAg sero-conversion and suppression of viral replication of HBV-DNA to less than 10^{4-5} copies/ml (7, 24, 27). Sustaining virological and biochemical responses has also been suggested as an additional treatment target (9, 34).

In the current study, the rate of HBeAg sero-conversion was not significantly different between the 3 groups at any time point. However, the HBeAg sero-conversion rate did increase with time during the first 12 months in the VAC and V+L groups. This result differed to that of the LAM monotherapy group, where a rapid increase in HBeAg sero-conversion was observed, suggesting that the vaccine slowly but steadily enhanced the immune response, thus controlling the clearance of HBV. Higher HBeAg sero-conversion rates have also been reported in the combination group when compared to the LAM monotherapy group after 12 months of treatment (12), further supporting our hypothesis. Additional studies using a greater number of participants are required to further support this hypothesis.

The current, randomized study resulted in an enhanced virological response in the combination group when compared to the vaccine and LAM monotherapy groups. The combination of vaccine and LAM was also found to be superior to the vaccine or LAM monotherapy alone in terms of early suppression (month 3-12), in the continuous control of HBV replication to HBV-DNA levels of < 4 log copies/ml or below the limit of detection, and in promoting a sustained virological response. However, the results obtained in our study differ to

those reported previously. Using 12 fortnightly doses of pre-S2/S vaccine, Horiike *et al.* reported that 9 (100%) HBeAg-positive patients receiving combination treatment demonstrated undetectable HBV-DNA levels (<3.7 log copies/ml) after treatment, but that only 15 of 31 patients (48%) in the LAM monotherapy group showed undetectable HBV-DNA levels (12). In contrast, using 12 doses of HBsAg/ASO_2 vaccine, Vandepapeliere *et al.* showed that viral suppression, as defined by HBV-DNA levels of < 5 log copies/ml, were not different between the combination and LAM groups during the study period (41). These variations in response are likely due to the differences in antigen composition of the vaccines, the vaccination schedules or the evaluation criteria of response in the two studies.

The mechanisms underlying viral clearance following therapeutic vaccination have been investigated in numerous studies. It is thought that specific exogenous antigens present in the vaccine may enhance the uptake and processing of HBsAg, recruit dendritic cells for antigen presentation around the injection site, up-regulate the major histocompatibility complex class II and CD86 on dendritic cells (1, 13), or up-regulate the production of IL-2, IFN-γ and TNF-α from antigen-stimulated T cells and differentiated lymphocyte T helper cells towards Th1 (18). The production of cytokines by the HBV-specific T lymphocytes may also reduce serum HBV-DNA levels via cytopathogenic and non-cytopathogenic pathways. These actions may be sufficient to suppress HBV-DNA levels, but not adequate enough to eradicate HBeAg. These findings suggest that the combination of vaccine and LAM may have more of an effect on viral load than HBeAg

sero-conversion rate. Additionally, combination treatment with vaccine may delay the appearance of LAM resistant mutations, and may help explain the steady increase in treatment response in the V+L group, but not the LAM monotherapy group. Further studies are required to investigate this hypothesis.

On the other hand, our results also demonstrate that the suppression effects were not well sustained after discontinued treatment with the HBV vaccine, results that are in agreement with previous reports (17). The fact that all patients in the combination group in our study continued to receive LAM, but not vaccine after 8 months indicated that the efficacy of viral suppression of the vaccine in the schedule was reduced when treatment with the vaccine was discontinued. Despite the rate of sustained HBV-DNA suppression after 9 months, and the higher rate of additional HBV-DNA suppression in the combination group as compared to the LAM monotherapy group, the rate of HBV-DNA breakthrough after 9 months was also found to be higher in the combination group, suggesting that the 8 injection schedule of vaccine therapy was not sufficient to cause a sustained response. Further studies may be required to optimize the immunization protocols for effective combination treatment in CHB patients.

Consistent with previous observations, we found that the number of patients exhibiting ALT normalization in the LAM monotherapy group was approximately 60-80% at the end of the study (10, 21, 26). A similar number of patients with biochemical response have also been reported in the 3 treatment groups at the end of the study, suggesting that biochemical response is

achieved by LAM monotherapy, vaccine monotherapy or V+L combination. At 18 months, biochemical response appears to be achieved without HBeAg sero-conversion or HBV-DNA suppression. However, patients in the vaccine monotherapy group normalized their ALT levels more slowly than the LAM monotherapy groups (21% vs. 55% after 3 months), suggesting that immune response to HBV after vaccine intervention contributed to the immune-induced pathogenic clearance of HBV during the first 12 months of treatment.

The HBsAg surface gene contains a neutralizing epitope termed α, which is located at codon position 124-147. Anti-HBs antibodies classically indicate viral clearance and life-long immunity after recovery from natural infection. Anti-HBs have been considered to be a key factor in clearance of virions and HBsAg-containing particles from the circulation (2), and are able to blockade viral particle receptors on new target cells (4). Hence, the co-existence of HBsAg and anti-HBs are generally not detected in natural infection. In the current study, anti-HBs were detected in 55/120 vaccine recipients (51.7% patients in combination and 31.7% in monotherapy groups at the end of the study), however only 4 patients also lost HBsAg during the study period. The remainder of vaccine recipients demonstrated co-existence of HBsAg and anti-HBs. Hence, the anti-HBs demonstrated in the study should be considered as vaccine-induced anti-HBs**.** The co-existence of HBsAg and anti-HBs was also observed in patients treated with a combination of HBV vaccine and LAM in Vandepapeliere's study (41), indicating that anti-HBs induced by therapeutic vaccination in CHB patients is not able to completely neutralize HBsAg in the serum,

or that a longer period of time is required for the complete removal of existing circulated HBV particles. Additional potential reasons for the ineffectiveness of anti-HBs on clearance of HBsAg may be that non-neutralizing epitopes are present on or exhibit a low sensitivity to anti-HBs, or the emergence of HBsAg mutants (20, 30). Mutations in the α region are thought to affect the neutralization of anti-HBs to their corresponding HBsAg, aiding escape of virus from the host immune system (44). In support of this hypothesis, a small number of HBV escape mutants have been reported in children following HBV immunization (14), however further analysis is required to confirm this possibility.

Interestingly, the current study also shows a higher rate of anti-HBs response in the LAM and vaccine combination groups compared to vaccine monotherapy, suggesting an interesting role for LAM in fostering responses following vaccine treatment. In contrast, vaccine recipients exhibiting a positive anti-HBs response demonstrate a greater probability of HBeAg sero-conversion and a higher HBV-DNA suppression, suggesting that anti-HBs may play an important role in enhancing HBV-DNA suppression in vaccine monotherapy and combination therapy.

In conclusion, our study suggests that the combined treatment with vaccine and LAM is more beneficial than LAM monotherapy in the short term, especially in terms of enhanced viral suppression and anti-HBs antibody response to vaccine.

Competing interests

The authors declare no competing interests either due to commercial or other affiliations.

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Figure legends

FIG. 1. Outline of the study design

FIG. 2. HBV-DNA reduction after 3 months of treatment. The percentage of patients with early HBV-DNA reduction (after 3 months) were expressed using two cut-off levels (reduced more than 1 log copy/ml and > 2 log copies/ml). The asterisk (*) indicates significant difference between V+L and LAM groups ($p<0.05$)

Figures

FIG. 1. Diagram of study design

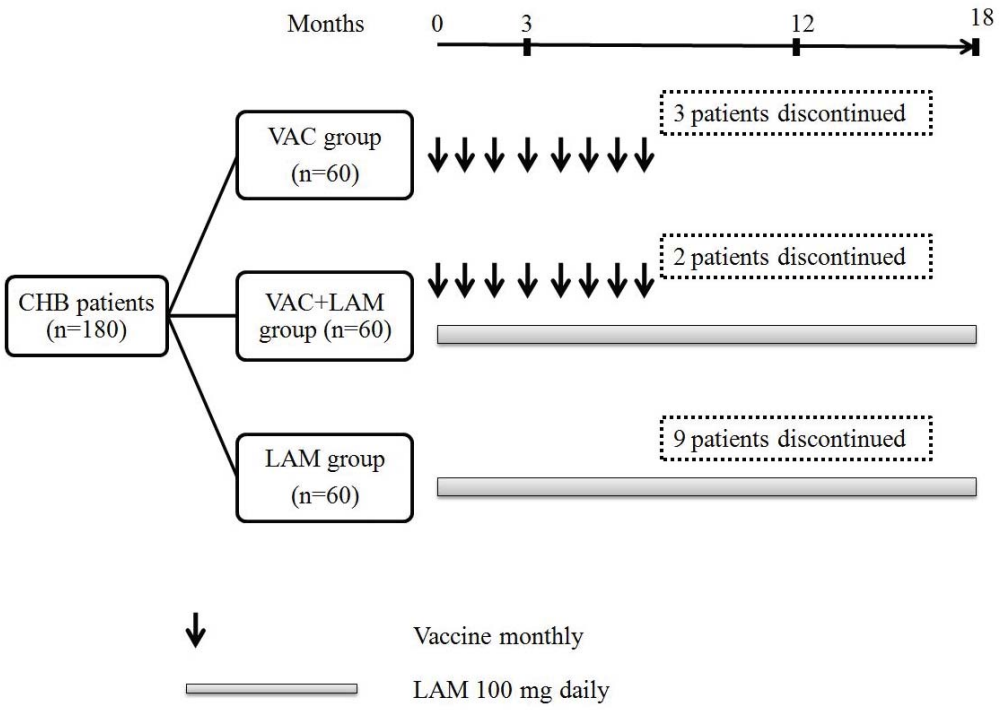

Fig. 1. Diagram of study design

FIG. 2. HBV-DNA reduction after 3 months of treatment

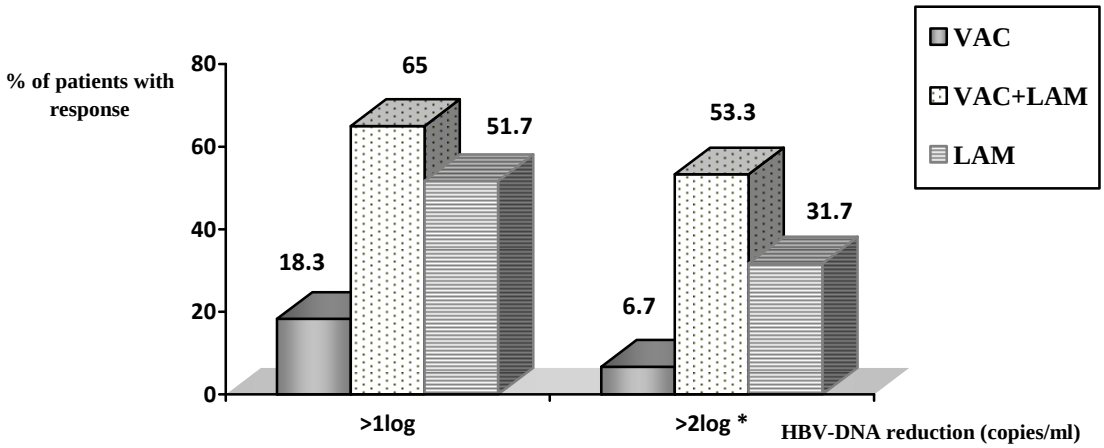

Fig. 2. HBV-DNA reduction after 3 months

(*p<0.05 for the comparison between VAC +LAM and LAM group)

TABLE 1. Baseline characteristics of the CHB patients

Characteristics		Total		Treatment group			p [a]
		n	%	VAC (%) (n=60)	V+L (%) (n=60)	LAM (%) (n=60)	
Gender	Male	114	63.3	65.0	65.0	60.0	NS
Age	<30	109	60.6	63.3	55.0	63.3	NS
BMI*	<24.5	161	89.4	85.0	90.0	93.3	NS
Previous LAM therapy	Yes	27	15.0	15.0	20.0	10.0	NS
Baseline HBV-DNA (log copies/ml)	>6	107	59.4	68.3	48.3	61.7	NS
	>5	149	82.8	83.3	78.3	86.7	NS
	>4	169	93.9	95	90	96.7	NS
	median	6.5		6.8	6.1	6.5	0.058[a,b]
	range			3.1→9.1	3.5→8.9	3.9→9.5	
Baseline ALT (ULN)	≤ 5	133	73.9	68.3	75.0	78.3	NS

n=180

*Body mass index.

[a] p values for the group comparison; NS: p>0.05

[b]Median test

TABLE 2. Serological and virological response in the 3 groups

Response & Time of evaluation	Treatment group			p*
	VAC n (%)	V+L n (%)	LAM n (%)	
After 3 months				
HBeAg loss	6 (10.0)	10 (16.7)	11 (18.3)	NS
HBeAg sero-conversion	4 (6.7)	7 (11.7)	11 (18.3)	NS
HBV-DNA reduction > 1 log copy/ml	11 (18.3)	39 (65.0)	33 (55.0)	<0.001
HBV-DNA reduction > 2 log copies/ml	5 (8.3)	32 (53.3)[a]	20 (33.3)[a]	<0.001 (0.027[a])
HBV-DNA ≤ 4 log copies/ml	5 (8.3)	33 (55.0)[a]	17 (28.3)[a]	<0.001 (0.044[a])
Median HBV-DNA reduction (log copies/ml)	0.0 (-3,16→3,75) (n=56)	-2,60 (-7,06→4,38) (n=54)	-1,9 (-7,09→2,23) (n=52)	***<0,001*** [b]
After 12 months				
HBeAg loss	18 (30.0)	14 (23.3	15 (25.0)	NS
HBeAg sero-conversion	16 (26.7)	14 (23.3)	11 (18.3)	NS
HBV-DNA ≤ 4 log copies/ml	9 (15.0)	39 (65.0) [a]	26 (43.3) [a]	<0.001 (0.017[a])
HBV-DNA ≤ 5 log copies/ml	19 (31.7)	44 (73.3)	39 (66.1)	<0.001
After 18 months				
HBeAg loss	21 (35.0)	18 (30.0)	17 (28.3)	NS
HBeAg sero-conversion	20 (33.3)	15 (25.0)	13 (21.7)	NS
HBV-DNA ≤ 4 log cp/ml	10 (16.7)	32 (53.3)	27 (45.0)	0.001
HBV-DNA ≤ 5 log cp/ml	20 (33.3)	35 (58.3)	35 (58.3)	0.02

*p values for the overall test of treatment effect.

NS: non-significant, p >0.05

[a] Significant difference between V+L and LAM groups.

[b] Median test.

TABLE 3. Biochemical response in the 3 treatment groups

Time of evaluation	Biochemical response (ALT < 1.5 ULN)			p[a]
	VAC n (%)	V+L n (%)	LAM n (%)	
3 months	13 (21.7)	23 (38.3)	33 (55.0)	<0.001
12 months	31 (50.0)	43 (71.7)	44 (73.3)	0.011
18 months	38 (63.3)	40 (66.7)	47 (78.3)	NS
[a]p values for the overall treatment effects.				

TABLE 4. The vaccine-induced anti-HBs response after 18 months for the 3 treatment groups

Month	Anti-HBs responders, n (%)			p[a]
	VAC	V+L	LAM	
3	2 (3.3)	3 (5.0)	0	NS
9	13 (21.7)	21 (35.0)	0	<0.001
12	17 (28.3) [b]	27 (45.0) [b]	0	<0.001
15	17 (28.3) [b]	28 (46.7) [b]	0	<0.001
18	19 (31.7) [b]	31 (51.7) [b]	0	<0.001
[a]p values for the overall treatment effects. [b]Significant difference of p<0.05 between VAC and V+L groups				

TABLE 5. Association between the vaccine-induced anti-HBs, serological and virological responses

	Vaccine induced anti-HBs after 18 months		p
	Yes (n=50) n (%)	No (n=70) n (%)	
HBeAg sero-conversion VAC V+L	20 (40.0) 11 (57.9) 9 (29.0)	15 (21.4) 9 (22.0) 6 (20.7)	0.027 0.006 0.45
HBV-DNA suppression (<4 log copies/ml) VAC V+L	28 (56.0) 7 (36.8) 21 (67.7)	14 (20.0) 4 (9.8) 10 (34.5)	<0.001 0.027 [a] 0.01
Median of HBV-DNA reduction (log copies/ml)	4.13 ± 2.9	5.52 ± 2.7	0.027
[a] Fisher's Exact test			

TABLE 6. HBeAg sero-conversion at month 18 compared to that at month 9

Response	HBeAg sero-conversion after 18 months, n (%)			p
	VAC	V+L	LAM	
Sustained or additional sero-conversion	20 (33.3)	15 (25.0)	13 (21.7)	NS [a]
No Response	40 (66.7)	41 (68.3)	44 (73.3)	
HBeAg reversion	0 (0.0)	4 (6.7)	3 (5.0)	
NS: not significant (p>0.05) [a] Fisher's Exact test				

TABLE 7. Virological response at month 18 compared to that at month 9 (Off-treatment response)

Virological response (HBV-DNA < 4 log copies/ml)	Treatment groups cases (%)			p
	VAC	V+L	LAM	
Sustained or additional HBV-DNA suppression	11 (18.3)	31 (51.7)	26 (43.4)	<0.001[a] 0.04[b]
Virological non-response	46 (76.7)	17 (28.3)	29 (48.3)	
HBV-DNA breakthrough	3 (5.0)	(20.0) [b]	5 (8.3) [b]	
[a]Comparison between the 3 groups; [b]Comparison between the V+L and LAM groups				