

Original Paper

Recombinant Hepatitis B Vaccine Adjuvanted With AS04 in Dialysis Patients: A Prospective Cohort Study

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Key Words

Adjuvant • Hepatitis B vaccine • Immunogenicity • Safety • Dialysis

Abstract

Background/Aims: Patients undergoing maintenance dialysis have an unsatisfactory response to vaccination, including to hepatitis B vaccine. A recombinant HB vaccine containing a new adjuvant system AS04 (HBV-AS04) has been recently developed; a few data exist on the immunogenicity and safety of HBV-AS04 among patients undergoing regular dialysis. All hepatitis B virus-seronegative patients with undetectable antibody against HBsAg undergoing maintenance dialysis at two units were prospectively included. **Methods:** Patients received four 20-mcg doses of HBV-AS04 by intramuscular route (deltoid muscle) at months 0,1,2, and 3. Anti-HB surface antibody concentrations were measured at intervals of 1, 2, 3, 4, and 12 months. Univariate and multivariate analyses determined which parameters predicted immunologic response to HBV-AS04 vaccine. **Results:** 102 patients were enrolled and 91 completed the study. At completion of the vaccination schedule, using per-protocol analysis, 76 of 91 (84%) had antibody titers ≥ 10 mIU/mL with anti-HBs geometric antibody concentrations (GMCs) of 385.25 mIU/mL. The sero-protection rate at month 12 was 84% (48/57) with lower GMCs (62.74 mIU/mL, $P < 0.0001$). Multivariate analysis revealed a detrimental role of age on the immune response to HB-AS04 vaccine (F Ratio, 4.04; $P < 0.04$). Tolerance to HBV-AS04 was good and only minor side-effects were observed. **Conclusions:** HBV-AS04 vaccine was highly immunogenic in our cohort of patients on maintenance dialysis even if a significant number of non-responders is still present. Prospective studies with HBV-AS04 on larger study groups and with longer follow-ups are under way.

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Introduction

Hepatitis B virus (HBV) infection is a serious global health problem with more than 350 million chronic carriers. Hepatitis B is a highly transmissible blood-borne virus, which leads to acute and chronic hepatitis, cirrhosis, hepatocellular carcinoma and liver failure. The frequency of hepatitis B infection is higher among dialysis patients than in the general population because of their constant exposure to blood, frequent transfusions, and sharing of dialysis equipment [1]. The relative stability of hepatitis B virus, which can remain infectious for up to 7 days at room temperature helps the spread of HBV within dialysis units.

The implementation of routine screening of blood derived products, and infection control practices including universal precautions, separate rooms and separate machines for HBsAg positive patients have sharply reduced the incidence of HBV infection in dialysis units [2, 3], even if outbreaks continue to occur in chronic haemodialysis centers [4, 5]. In addition, HBV vaccination has been recommended for all sero-negative dialysis members and staff members since the early 1980s [2, 3]. However, patients undergoing long-term dialysis have a poor immunologic response to hepatitis B vaccine in comparison with healthy individuals- the immunisation rate and the anti-HBs titers are lower after completion of the vaccination schedule and fall logarithmically. Various approaches have been used to improve the immunogenicity of second-generation (or recombinant) HB vaccine in chronic uraemia, including reinforced HB vaccine schedules [6, 7], concomitant use of immuno-modulators [8, 9], intradermal administration of HB vaccine [10], and vaccination of chronic kidney disease patients at pre-dialysis stage [11], among others. A recombinant HB vaccine containing a new adjuvant system (AS04) has been recently developed but the evidence on the immune response of HBV-AS04 in dialysis population is limited [12, 13]. HBV-AS04 is a novel adjuvant system containing aluminium salt and 3-*O*-desacyl-4-*_*-monophosphoryl lipid A (MPL); it has been suggested that the adjuvant system AS04 could stimulate cellular and humoral responses via an increased antigen-presenting capacity through upregulation of the CD86 molecule and/or via an increased synthesis of cytokines.

The aim of this prospective, cohort study was to expand our knowledge on the efficacy, and safety of adjuvanted recombinant vaccine in patients with chronic kidney disease. We have administered HBV-AS04 to a large cohort of susceptible patients undergoing maintenance dialysis at two urban dialysis units.

Patients and Methods

Setting and study subjects

This was a prospective, cohort study in outpatient patients receiving long-term dialysis in two dialysis units in Milano city. There was a program-wide screening for hepatitis B virus infection susceptibility and immunization at the time of this study. Hepatitis B virus-susceptible patients were given an option to participating in the study.

Inclusion criteria were receiving long-term dialysis treatment (haemodialysis or peritoneal dialysis), 18 years or older, serum HBsAg/ anti-HBs negative at baseline, and able and willing to give informed consent. Exclusion criteria were treatment with intravenous immune globulin within the last 6 months, previous allergic reactions including hypersensitivity to components of vaccine, contraindication to intramuscular injections, elevated liver enzymes, serious systemic illness, and simultaneous immunosuppressive therapy. Patients with previous administration of a vaccine containing mono-phosphoryl lipid A were excluded. Standard haemodialysis techniques were performed with 3- or 4-hour treatments three times a week; blood and dialysate flow rate of 300 and 500 mL/min, respectively, were used in order to satisfy the criteria of adequacy based upon urea kinetic analysis. All patients on peritoneal dialysis underwent continuous ambulatory peritoneal dialysis with three or four exchanges daily.

The study was conducted in accordance with the ethical principles reported in the 1996 version of the Declaration of Helsinki and Good Clinical Practice guidelines. All participants gave informed and written consent before they were enrolled. The study was reported according to the STROBE initiative [14]; the

checklist of 22 items that it is considered essential for good reporting of cohort observational studies is freely available on the STROBE web site at <http://www.strobe-statement.org/>.

Study design

Patients were given four doses of recombinant vaccine formulated with a new adjuvant system (HBV-AS04, Fendrix™) manufactured by GlaxoSmithKline Biologicals. HBV-AS04 vaccine was administered as a 0.5-mL intramuscular injection (deltoid muscle of the arm without the haemodialysis artero-venous fistula) according to a 0-, 1-, 2-, 3- month schedule. One dose of HBV-AS04 contained 20 mcg of recombinant HBsAg, 50 mcg of MPL (3-0-desacyl-4'-monophosphoryl lipid A) and 0.5 mcg of aluminium salt. The primary outcome of interest in this study was the sero-protection rate in the ATP (According to Protocol) cohort, as a measure of efficacy. The ATP cohort included all patients who had complied with primary vaccination protocol. The secondary end-point was the frequency of side-effects associated with vaccine administration.

Serum studies

Upon entry into the study, a baseline serum sample was obtained. Samples for anti-HBs antibodies were taken from all study patients at intervals of 1, 2, 3, 4, and 12 months. HBV, HCV and HIV markers were tested in all patients at baseline. Hepatitis B surface antigen (HBsAg); antibodies to hepatitis B surface antigen (HBsAb) and hepatitis core antigen (HBcAb) were measured in plasma samples by enzyme immunoassays (Abbott Laboratories, USA). Serum amino-transferase levels were tested with spectrophotometric method. Screening for antibody to hepatitis C virus (HCV) was performed by a third-generation ELISA test. Antibodies to the human immunodeficiency virus were measured by commercially test kits (Abbott Diagnostics).

Safety and reactogenicity

The patients were observed closely for at least 15 minutes after each vaccination. The safety and reactogenicity were evaluated by the patients recording solicited local (pain, redness, swelling) and general (fatigue, fever, headache, and gastrointestinal symptoms) adverse events. All local symptoms were considered as related to vaccination. Unsolicited adverse events occurring within 30 days after each vaccination regardless of attribution were also recorded as well as any serious adverse event (AE) that occurred during the whole study period up to 30 days after the last vaccination.

Statistical analysis

Descriptive analyses are expressed in terms of mean± standard deviation for continuous variables and percentages for categorical variables. Skewed variables were log-transformed. We performed multivariate analysis by standard least squares model: age, gender, ethnicity, time on dialysis, type of dialysis treatment (haemodialysis or peritoneal dialysis), anti-HCV serologic status, patient allocation among the dialysis units, diabetes mellitus, anti-HBc serologic status, prior vaccination history, haemoglobin concentration; and serum transferrin were used as independent variables, and anti-HBs titers were assumed as dependent variables. Statistical analysis was performed using the program JMP (®SAS Institute, USA, 1996).

Definitions

Successful vaccine response (sero-protection) was defined as an anti-HBs concentration ≥ 10 mIU/mL. Sero-conversion was an anti-HBs value of 1 mIU/mL or more. We defined responder and non-responder patients according to the level of anti-HBs one month after the final injection (non-responders, <10 IU/L; responders, ≥ 10 IU/L).

Results

Patient characteristics

We originally enrolled 102 patients, 91 of them completed the study, for which per-protocol analysis was made. Some patients died ($n=8$), or withdrew from the study on a voluntary basis ($n=2$), or were lost during the study period ($n=1$) and did not complete fully the vaccine schedule.

Shown in Table 1 are some salient demographic and clinical characteristics of subjects enrolled in the study. The majority of our patients had Caucasian origin, were naïve, and underwent maintenance haemodialysis. Twelve (13.1%) were non responders to a prior course of recombinant vaccine (Engerix-B). Eighteen (19.7%) underwent dialysis at unit 2 and 73 (80.3%) at unit 1. Twenty-one (23%) patients were in the active waiting list for renal transplant. No patient had detectable human immunodeficiency virus antibodies or admitted a history of intravenous drug use.

Immunogenicity of the vaccine

After the first vaccine dose, the sero-protection rate was 34% (11/32) (Fig. 1). The sero-protection rate after the second vaccine dose was 54% (26/48). The sero-protection rate after the fourth dose and at 12 months was 84% (76/91) and 84% (48/57), respectively. GMCs after the fourth dose were greater ($P < 0.0001$ for any comparison) than at other times. No significant difference occurred concerning GMCs at month 1, 2, 3, or 12; ($P = NS$), (Figure 1). The baseline characteristics of responders

Table 1. Baseline characteristics of study patients

		Patients, n	(%)
Gender	Male	44	(48.4)
	Female	47	(51.6)
Age (years)	Mean (SD)	67.2 (14.9)	
	Median	71.0	
	Range	31 to 91	
Ethnicity	Caucasian	83	(91.2)
	Others	8	(8.8)
Underlying nephropathy	Polycystic kidney	9	(9.9)
	Diabetic nephropathy	9	(9.9)
	Glomerulonephritis	16	(17.6)
	Urolithiasis	6	(6.6)
	Nephrosclerosis	27	(29.7)
	Unknown cause/others	20	(22)
	Neoplasia	4	(4.3)
Dialysis type	Hemodialysis	83	(91.2)
	Peritoneal dialysis	8	(8.8)
Vaccine status	Naïve	79	(86.8)
	Experienced	12	(13.2)
Dialysis Vintage (months)	Mean (SD)	37.6 (39.7)	
	Median	24.0	
	Range	3 to 178	
Diabetes mellitus	Yes	24	(26.4)
	No	67	(73.6)
Anti-HCV serologic status	Positive	8	(8.8)
	Negative	83	(91.2)
Anti-HBc serologic status	Positive	8	(8.8)
	Negative	83	(91.2)
Hemoglobin (g/dL)	Mean (SD)	11.02±0.99	
	Median	11	
	Range	8.3 to 13.9	
Transferrin (mg/dL)	Mean (SD)	189.4±119.7	
	Median	187	
	Range	133 to 293	

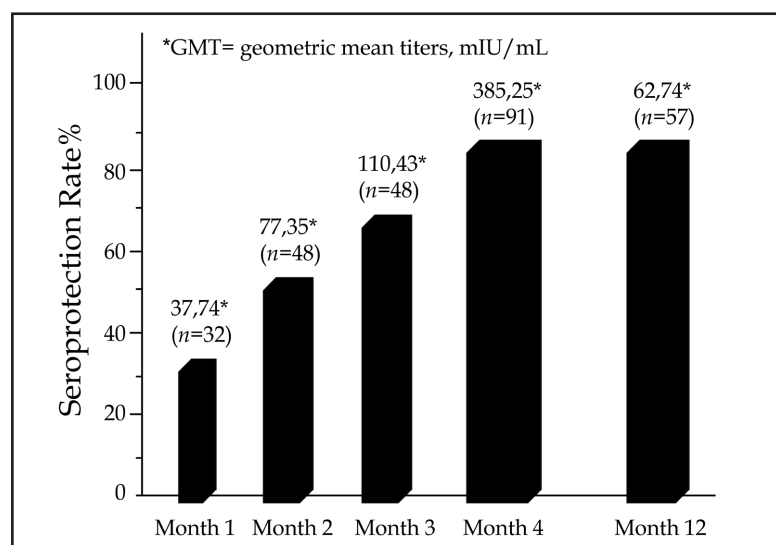


Fig. 1. Serial sero-protection rates and geometric mean times over follow-up among dialysis patients. After the first and the second vaccine dose, the sero-protection rate was 34% and 54%, respectively. The sero-protection rate after the fourth dose and at 12 months was 84% and 84%, respectively. The greatest levels of geometric mean titers were at month 4 (385, 25 mIU/mL).

and non-responders patients have been shown in Table 2. In the subset of patients who underwent revaccination with HBV-AS04 because non-response, the sero-protection rate was 91.7% (11/12), even if no significant difference occurred (Table 2).

As listed in Table 3, multivariate analysis showed the negative impact of age on the sero-protection rate ($P=0.041$) whereas no link between sero-protection rate and other demographic or clinical parameters was found.

Safety of the vaccine

As reported in Table 4, many patients experienced AEs (47/91 = 51.6%). The majority of the side-effects were injection-site AEs; the most common being pain (30/91=33%). The most frequently reported vaccine-related systemic AEs were headache and fever (6/91=6.6%). Overall, eight deaths occurred. None of the deaths were considered to be related to vaccine by the investigators.

Discussion

First-generation (plasma-derived) hepatitis B vaccines had been developed in the US and western Europe in the late 1970s. Since the mid 1980s, second-generation recombinant hepatitis B vaccines have gradually replaced the plasma-derived vaccines even among patients with CKD. Second-generation vaccines have been made in yeasts transfected with HBV-DNA sequences coding for the small hepatitis B surface protein (SHBs) [15]. They are currently used for universal vaccination of newborns and adults in >170 countries worldwide. Despite their extraordinary efficacy second-generation HB vaccines can result in immunization failure which can be explained by numerous variables including renal failure and immuno-suppression. Various options are currently available to address immunization failure in patients with end-stage renal disease; second-generation recombinant vaccines formulated with adjuvants [12, 13, 16], or manufactured by a modified process [17] or third-generation HB vaccines [18] look promising.

Table 2. Baseline characteristics of responder and non-responder patients

	Responders (n=76)	Non responders (n=15)	P
Gender, males	34 (44.7%)	10 (66.6%)	NS
Age, yrs	65.6±13	75.4±13	0.01
Ethnicity, Caucasians	68 (89.4%)	15(100%)	NS
Diabetes, n	21 (27.6%)	3 (20%)	NS
Anti-HCV, n	7 (9.2%)	1 (6.6%)	NS
Anti-HBc, n	5 (6.5%)	3 (20%)	NS
Experienced, n	11 (14.4%)	1 (6.6%)	NS
CAPD, n	8 (10.5%)	0	NS
Time on dialysis, mo	38±41	35.8±33.6	NS
HB, g/dL	11±1.04	10.8±0.7	NS
Transferrin, mg/dL	190.5±33	184.1±37	NS

Table 3. Multivariate analysis by standard least squares: Parameter estimates and effect test

Source	N- parm	DF	Sum of squares	F Ratio	Prob>F
Gender	1	1	4031,5119	3,789	0,055
Ethnicity	1	1	1,9571	0,0018	0,96
Age, yrs	1	1	4302,0223	4,04	0,04
Naïve, pts	1	1	305,1701	0,2868	0,593
Dialysis mode	1	1	360,8300	0,3391	0,5619
Diabetes	1	1	2227,7853	2,0939	0,1511
Anti-HBc status	1	1	2493,4166	2,2844	0,1347
Time on dialysis	1	1	2105,2696	1,9288	0,168
Location	1	1	79,8293	0,0731	0,787
Anti-HCV	1	1	1382,9260	1,2670	0,2638
Hb	1	1	160,5538	0,1471	0,7024
Transferrin	1	1	163,8539	0,1501	0,6995

In this prospective, cohort study we have evaluated the reactogenicity and immunogenicity of a second-generation HBV vaccine adjuvanted with AS04 in patients on regular dialysis; the sero-protection rate was 84% (76/91). Such immunologic response was lower than that observed by Tong *et al.* in their Fendrix arm (91%) [12]. The study by Tong *et al.* was an open, randomized clinical trial which selected chronic kidney disease patients according to strict criteria, and it is well established that an universal flaw of RCTs is their limited external validity [19]. We have enrolled in the current study an unselected cohort of patients on maintenance dialysis, with a high proportion of CKD-associated co-morbidities (arterial hypertension, diabetes, anaemia, and cardiovascular disease) and long dialysis vintage; these characteristics are consistent with daily clinical activity.

We have used an accelerated vaccine regimen (HBV-AS04, 0, 1, 2, and 3 months) compared with the standard schedule used by Tong *et al.* and recommended by the manufacturers (HBV-AS04; 0, 1, 2, and 6 months). This precludes definitive comparisons on the immunogenicity of recombinant vaccine adjuvanted or not with AS04. Our accelerated schedule has given us the possibility to obtain sero-protection as soon as possible to our susceptible patients.

The data in the published literature on the immunogenicity and tolerance of HBV-AS04 are limited not only in chronic kidney disease population. Preliminary data exist on adjuvanted HBV-AS04 use among liver transplant candidates [20] and HIV-infected patients [21, 22]. Previous evidence has shown a high immunogenicity profile of adjuvanted HB-AS04 among healthy individuals [23].

A major finding of the present study was the evaluation of predictive factors which could play impact on the immune response to HBV-AS04. Age was an independent predictor of immune response to HBV-AS04 in our multivariate analysis. We have previously found a significant relationship between age and sero-protection rate after primary vaccine course with standard recombinant vaccine- mean age being greater in 'transient' than 'persistent' responders after a primary vaccine course, 72.2 vs. 61.3 years ($P < 0.003$) [6]. The relationship between lowered immune response towards HB vaccine and ageing has been already noted in other reports [24, 25] and is well-known among patients with intact kidney function [26].

In the current study, we reported no interaction between gender, type of dialysis, time on dialysis, anti-HCV antibody rate, diabetes mellitus, HB levels, serum transferrin and the immune response to HBV-AS04. We included serum transferrin as a surrogate marker of nutritional status. That is partially in contrast with other studies based on plasma-derived or standard recombinant vaccine. We cannot exclude residual confounding as the role of additional factors including inadequate dialysis, erythropoietin deficiency, smoking, quality of life [27], body mass index, inflammatory status, and iron loading on the immune response

Table 4. Adverse experience summary (days 1 to 30 following any vaccination visit)

	Patients (n=91)	(%)
Patients with one or more AE	47	(51.6)
Patients with injection-site AEs	41	(45)
Erythema	6	(6.6)
Pain	30	(32.9)
Swelling	5	(5.5)
Systemic AEs	6	(6.6)
Headache	4	(4.4)
Fever	0	(0)
Asthenia	2	(2.2)
Gastro-intestinal symptoms	0	(0)
Patients with serious AEs	8	(8.8)
Serious vaccine-related AEs	0	0
Pts who died	8	(8.8)
Patients discontinuing vaccine due to AEs	0	0
Maximum temperature (Days 1-5 post-vaccination)		
>37.5	0	0
>40	0	0

after HBV-AS04 vaccine was not investigated. An additional shortcoming of this study was that the evaluation of the decay rate of anti-HBs titers following initial vaccination with HBV-AS04 was made over a short follow-up (12 months); active research on this point exists [28]. Abundant literature on the immune response to plasma-derived or conventional recombinant vaccine (Engerix-B) suggests that the protection against hepatitis B in patients with immune compromise does not rely on immune memory (in the form of HBsAg-specific memory lymphocytes) but on circulating anti-HBs antibodies [29].

Various benefits have been linked to the vaccination against HBV in patients with chronic kidney disease including the possibility that HBsAg negative patients with sero-protective levels of antibody against HBV (HBsAb ≥ 10 mIU/mL) can undergo haemodialysis in shifts and rooms dedicated for HBsAg positive patients. Isolation of HBsAg positive carriers by rooms, machines, and staff remains an important preventive measure against the spread of HBV within dialysis units, as recommended by the CDC [3]. Sero-protection against HBV is crucial to RT candidates in order to be included in the waiting list from anti-HBc positive kidney donors. Also, vaccination towards HBV in patients with CKD is an effective measure to reduce the progression of chronic kidney disease as a significant link between HBsAg positive serologic status and increased risk for end-stage renal disease has been recently noted [30].

Conclusion

In summary, our prospective cohort study suggests a relevant number of non-responder dialysis patients who have completed vaccination with HBV-AS04. Novel data support a link between infection-cause mortality and poor immune response to HB vaccine in end-stage renal disease [31]. Prospective studies with third-generation vaccines, or conventional recombinant HB vaccine provided with alternative adjuvants or immuno-stimulants are in progress to improve results of hepatitis B vaccination in chronic kidney disease population.

Abbreviations

AE, adverse events; CAPD, continuous ambulatory peritoneal dialysis; CDC, Centers for Disease Control and Prevention; CI, confidence intervals; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GMC; geometric mean titers; HBV, hepatitis B virus; RCTs, randomized clinical trials; RT, renal transplant

Disclosure Statement

FF and PGM received fees (consultant) from GlaxoSmithKline Biologicals. No conflicts of interest were declared by the other authors.

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