Turk J Gastroenterol 2002; 13 (1): 6-10

The effect of recombinant hepatitis B vaccine therapy in chronic hepatitis B infection

Kronik hepatit B enfeksiyonunda rekombinan hepatit B aşı tedavisinin etkisi

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Background/aims: *Immune-modulator and antiviral treatments for carriers of hepatitis B virus are known to have poor efficacy with a high cost and frequent side effects, which has led to investigation of new treatment modalities. The aim of this study was to determine the efficacy of recombinant hepatitis B vaccine in the treatment of patients with chronic hepatitis B (HBV DNA>5 pg/ml, ALT>60) infection diagnosed histopathologically and asymptomatic carriers (HBV DNA<5 pg/ml, ALT <40, Anti Hbe positive) of the virus.* ***Methods:*** *The vaccine (Gen Hevac B Pasteur) was administered at baseline and at months one and six to patients with chronic hepatitis and asymptomatic carriers. Ten cases with chronic hepatitis B infection were assigned to a control group to whom no treatment was given. Biochemical and microbiological investigations were performed at baseline and at months three, six and twelve in all cases. Seroconversion of Hbe Ag, loss of HBV DNA and normalization of ALT were considered as a positive response.* ***Results:*** *Patients with chronic hepatitis B who were given the vaccine were found to have significantly low levels of HBV DNA at 12 months (63.2±20pg/ml) compared to baseline values (174.4±36.9pg/ml), while controls were found to have high levels of HBV DNA at 12 months (223.1±33pg/ml) compared to baseline values (165.2±33.2pg/ml) (p<0.05). At 12 months, HBV DNA had become negative in seven of 19 patients given the vaccine (36.8%) Four patients with chronic hepatitis (36.35%) were observed to have HBeAg seroconversion and one patient (5.2%) HBsAg seroconversion at the end of 12 months and there were four (21.05%) patients who responded positively to vaccine therapy in this group. Asymptomatic carriers and controls did not have seroconversion of HBs Ag. Also, HBV DNA did not become negative in controls.* ***Conclusion:*** *It may be concluded that recombinant hepatitis B vaccine is effective in the treatment of chronic hepatitis B.*

Key words: Chronic hepatitis B, healthy hepatitis B virus carrier, recombinant hepatitis B vaccine.

Amaç: *Kronik hepatit tedavisinde denenen immünmodülatör ve antiviral tedavilerin etkinliklerinin istenen düzeylerde olmaması, bu tedavilerin yüksek maliyeti ve sık görülen yan etkilerinden dolayı HBV taşıyıcıları için tedavi arayışları sürmektedir. Bu çalışmanın amacı HBV enfeksiyonu sonrası kronik hepatit gelişen veya taşıyıcı olan olguların tedavisinde rekombinan hepatit B (rHB) aşı uygulamasının etkinliğini belirlemektir.* ***Yöntem:*** *Kronik hepatit tanısı alan 19 ve asemptomatik taşıyıcı olan 18 olguya 0,1 ve 6. ay olmak üzere üç defa 0.5 cc (Gen Hevac B Pasteur) rHB aşısı uygulandı. Kontrol grubu olarak kronik hepatit B tanısı alan 10 olgu tedavisiz izlendi. Olguların 0, 3, 6 ve 12. ay da biyokimyasal, mikrobiyolojik tetkikleri ve HBV DNA düzeyleri çalışıldı. Hbe Ag serokonversiyonu, ALT normalleşmesi ve HBV DNA' nın kaybolması durumunda tedaviye pozitif cevap oluştuğu kabul edildi.* ***Bulgular:*** *Kronik hepatitli olguların 12. ay (63.2±20 pq/ml) HBV DNA düzeylerinin 0. ay (174.4±36.9 pq/ml) düzeylerinden anlamlı düşük olduğu kontrol grubunda ise 12. ay (223.1±33 pq/ml) HBV DNA düzeylerinin 0. aydan (165.2±33.2 pq/ml) anlamlı yüksek olduğu gözlendi (p<0.05). Çalışma grubunu oluşturan 19 olgunun yedisinde HBV DNA' nın (%36.8) negatifleştiği, ALT' nin normal düzeye indiği gözlendi. Kronik hepatitli olguların dördünde (%36.35) HBeAg serokonversiyonu, birinde ise (%5.2) Anti HBs oluşumu gözlendi. Bu olguların dördünde (%21.05) pozitif cevap oluştuğu gözlendi. HBV DNA' nın negatif olduğu taşıyıcı olgularda ve kontrol grubu olgularda HBs Ag serokonversiyonu gözlenmedi.* ***Sonuç:*** *Çalışmamız sonuçlarına göre rHB aşısı kronik hepatit B tedavisinde etkili bir tedavi yöntemi olarak düşünülebilir.*

Anahtar kelimeler: Kronik hepatit B, asemptomatik hepatit B taşıyıcılığı, rekombinan hepatit B aşısı.

INTRODUCTION

Hepatitis B, a non-cytopathic virus, infects the liver cells and cellular and humoral immune responses determine the disease outcome (1). Five percent of cases develop chronic hepatitis following infection and in time face an increased risk of cirrhosis and hepatocellular carcinoma (HCC) (2). Efficacy of the most satisfactory combinations of immuno-modulating and antiviral treatments has been around 30-45% in chronic hepatitis (3-6). Furthermore, these treatment regimes are costly and cause frequent and serious side-effects, which has lead to increasing efforts to find new treat-

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Manuscript received: 26.6.2001 **Accepted:** 22.1.2002

ment modalities (7). In addition, the treatment of asymptomatic hepatitis B carriers is of concern due to periods of flares and the potency of cirrhosis and HCC in this group of patients (8,9).

Recombinant hepatitis B vaccines have long been used for protection in the serum of and three doses have been shown to produce Anti HBs in the serum of approximately 95% of people who have not encountered the virus (10). Two kinds of recombinant vaccine are used for active immunization against hepatitis B; one of them contains the PreS1 and PreS2 antigenic domains while the other kind contains S and PreS polypeptide. No important differences between the effectiveness of these two types of vaccine have been detected (11). Recently, vaccine therapy has been proposed for the treatment of some chronic hepatitis B viral infections (12,13).

The aim of this study was to determine the efficacy of recombinant hepatitis B (rHB) vaccine in patients with chronic hepatitis B and asymptomatic hepatitis B carriers

MATERIALS AND METHODS

Patients

This study included 19 patients diagnosed with chronic hepatitis (Group I), 18 asymptomatic carriers (Group II) and 10 patients diagnosed with chronic hepatitis (controls) who were referred to Internal Disease Outpatient Clinics of Mersin University Hospital, Turkey, between 1999 and 2000. The patients with chronic hepatitis were positive for HBs Ag and HBV DNA and their ALT values were 1.5 times higher than the normal values. The asmyptomatic carriers of hepatitis B virus were negative for HBV DNA, positive for Anti HBe, positive for HBs Ag and had normal level of ALT (<40). The controls were positive for HBs Ag and HBV DNA and their ALT levels were 1.5 times higher than the normal.

Study Protocol

All patients and carriers underwent physical examination, biochemical examination and investigations of hepatitis markers (HBs Ag, Anti HBs, HBe, Hbc IgM, Anti HCV) and HBV DNA. Biopsy of the liver was performed under ultrasonography using Tru-cut biopsy needle and the material obtained was evaluated by the same pathologist according to Knodell's histologic activity index (14). Patients with Anti HCV antibody and Anti HIV antibody, with alcohol dependency, previously treated for chronic hepatitis and those found to have cirrhosis on biopsy were excluded from the study. The vaccine Gen Hevac B Pasteur (contains S and PreS polypeptide) 0.5 ml was administered by intramuscular injection into the deltoid region of patients and carriers in both study groups at baseline and at months one and six. Control patients were not given any treatment. Biochemical analyses and investigations of hepatitis markers and HBV DNA were performed at baseline and three, six and 12 months. On the basis that antibody synthesis starts two weeks after vaccination (15), biochemical assessments were made 15 days after the third vaccination. A positive response to treatment was defined at the end of one year as loss of serum HBV DNA (<5 pg/ml), seroconversion from HBe Ag (if present) to Anti HBe and normalization of ALT level (<40)

Viral Markers

Measurement of hepatitis markers were made in accordance with random access Elisa system using Elisa kits (Abbott USA) and AXYM device. Cut-off points for HBs Ag, HBe Ag, Anti HBe and Anti HCV were considered 2, 10, 1, <1 and 1 respectively.

HBV Hybridization assay

HBV DNA solution hybridization was carried out in the sera of patients using appropriate kits and with probes of HBV pointed by I 125 (Diagen/USA). A set of oligonucleotide probes bind single-stranded HBV DNA to a solid phase, which is detected by a second set of oligonucleotide probes. The assay protocol was according to the manufacturer's instructions. Briefly, 10 µl of test samples or HBV DNA-positive standards (0.5 to 4,400 MEq/ml) was added to 10 µl of lysis reagent on a microwell plate coated with oligonucleotide probes and incubated for 30 min at 63°C to release HBV DNA from the viral particles. Ten microliters of the second set of probes in denaturing buffer was then added to the wells and incubated for 30 min at 63°C. Ten microliters of neutralizing reagent was then added, and the hybridization process continued for 16 hours. After washing, amplifiers were added and incubated for 30 min at 53°C. After washing, 30 µl of dioxetane substrate was added. The plate was incubated for 25 min at 37°C in the Chiron luminometer, and light emission was measured for each well (16).

Statistics

Man Whitney U, One Way Anova and Chi-square tests were used for statistical analysis. $P<0.05$ was considered significant.

RESULTS

Table 1. Results of ALT, HBV DNA, seroconversion of HBe Ag and HBs Ag in study and control groups.

	0. months		6. months			12. months			
	ALT	*HBV DNA*	*ALT*	*HBV DNA*	*HBe Ag Seroconversion*	*ALT*	*HBV DNA*	*HBe Ag Seroconversion*	*HBs Ag Seroconversion*
GroupI (n:19)	63.5±4	174.4±36	36.0±3	79.8±5	2	36.4±3	63.2±20	4	1
Group II (n:18)	32.7±2	0	28.2±1	0	0	31.5±2	0	0	0
Control (n:10)	64.1±5	165.2±33	61.3±3	158±24	0	72.9±7	223±33	0	0

Age and sex were comparable in study groups I (12 female, seven male; age: 36±3.4 years) and II (10 female, eight male; age: 41±2.5 years) and in the control group (six female, four male; age: 39.2±8.2 years). At baseline, there was no difference between ALT levels in Group I (63.52±4.18U/L) and the control group (64.18±5.25U/L) but when these two groups were compared with the Group II (32.77±1.97U/L), the difference was significant ($p<0.05$). At baseline and 12 months there was significant decrease in ALT levels of Group I ($p<0.05$) while in the control group, ALT level was 64.18±5.35U/L at baseline and 72.9±7.35U/L at 12 months ($p<0.05$) (Table 1).

In Group I, anti HBe was positive in eight cases (42%) at baseline, in ten cases (52%) at six months and in 12 cases (63%) ($p<0.05$) at 12 months. Apart from positive Anti HBe cases at baseline, four cases (36.35%) were found to have seroconversion from HBe Ag to anti HBe. Anti HBs was negative in all cases at baseline and there was no change at three and six months, but Anti HBs antibodies (>100U/L) were detected in one case (5.2%) at 12 months. In five of the controls (50%), HBe Ag was positive and in the remaining Anti HBe was positive at baseline, but no change was detected in serological tests at three, six and 12 months (Table 1).

There was no significant difference in baseline HBV DNA levels between the Group I (174.47±36.93pg/ml) and control patients (165.27±33.25pg/ml) ($p>0.05$). However, the HBV DNA level at 12 months was significantly low (63.21±20.5319pg/ml) in Group I, while it was significantly high (223.10±33.8pg/ml) in the control group ($p<0.05$). HBV DNA was positive in all cases of Group 1 at baseline and negative in three cases (15%) at six months (Table 1). It was observed that HBV DNA disappeared in seven of 19 cases (three HBe Ag positive at baseline with no HBe Ag seroconversion at 12 months, two HBe Ag positive at baseline with HBe Ag seroconversion at 12 months and two Anti HBe positive) in Group I (36.8%) at 12 months, and that the ALT level of all these seven cases was below 40IU. Thus there were four patients (21.05%) who responded positively to vac-

Table 2. Comparison of ALT and HBV DNA levels of chronic hepatitis patients with an without seroconversion of HBe Ag.

		0. months		6. months		12. months	
	AGE	*ALT*	*HBV DNA*	*ALT*	*HBV DNA*	*ALT*	*HBV DNA*
Seroconversion of HBe Ag (n:4)	29.2±88	68±102	363.5±110.0	35±3.8	221.2±782	39.7±6.9	7±2.8
No Seroconversion of HBe Ag (n:7)	34.7±5.7	63±5.04	167.2±46.26	42.8±4	125.8±20.1	34.0±5.2	87.5±14.0
P value	0.62	0.69	0.04	0.46	0.081	0.52	0.03

cine therapy in Group I. There was no significant difference in mean ages, HAI and HBV DNA levels of cases who responded positively and those who did not. In the control group, HBV DNA did not become negative in any cases.

Comparison of the four cases with and seven cases without HBe Ag seroconversion showed no significant difference in age, HAI scores and ALT levels at baseline ($p>0.05$), although HBV DNA levels were higher in cases with seroconversion ($p<0.05$). Also, there was no significant difference in the mean age of cases responding to treatment, they were younger than those not responding to treatment. At 12 months, there was no significant difference in ALT levels between these two groups, but HBV DNA levels were significantly lower in cases with seroconversion of HBe Ag than those without ($p>0.05$) (Table 2). While low titres of HBV DNA were determined in one of the four cases with seroconversion of HBe Ag, HBV DNA titres of another was measured as 9 pg/ml. It was thought that this virus may develop mutations in the core gene.

DISCUSSION

In this study, HBV DNA became negative in 36.8% of patients and seroconversion from HBe Ag to AntiHBe occurred in 36.3% of patients with chronic hepatitis who received vaccine therapy; thus a positive response was determined in 21.05% of these patients while no seroconversion occurred in the other groups. Pol et al. reported that HBV DNA became negative in 55.6% of 46 patients six months after treatment with rHB vaccine given three times at monthly intervals (15). Couillin et al., in their series of 17 cases who were administered six doses of vaccine, noted that HBV DNA became negative in 57.1% of their cases, Anti HBe occurred in 29.4%, but HBs Ag seroconversion did not occur in any case at the end of 12 months follow up (17). Similarly, several other studies have reported that HBV became negative in almost half of patients, while HBs Ag seroconversion was not observed (18,19). Akbar et al reported that of 32 HBV transgenicmice, whose HBV DNA, HBe Ag and HBs Ag were positive and which were given 10µg of vaccine every month for 12 months, HBe Ag antibodies disappeared in 30 HBV transgenicmice (93.7%), Anti HBs antibodies developed in five HBV transgenicmice and HBV DNA levels significantly decreased in all rats (20). The results of all these studies show that the vaccine is an effective treatment modality. Although the findings of the present study are concordant with previous reports, the small number of cases limit the conclusions which may be drawn.

Despite advances in this field, the immunological pathophysiology implicated in the marked differences in the prognosis of chronic viral hepatitis B patients has not been fully understood (21). A virus causing infection in the liver should be recognized by CD4 TH cells, an important molecule of the immune system, and dendritic cells should sufficiently process the antigen for the recognition of the virus (22). TH1 cells, which are a subtype of CD4 TH, release interleukin-2 (IL-2) and INF-gamma (23), which have an inductive role in the synthesis of B lymphocytes with characteristics of Ig G1 and Ig G2. Anti HBs developing after hepatitis B virus infection have been shown to possess the nature of Ig G1 and Ig G2 (24). When cytokines were measured in healthy hepatitis B virus carriers and patients with chronic hepatitis following vaccination, INF-gamma levels were observed to increase significantly in those responding to treatment (25,26). Several investigators suggested that the functions of dendritic cells were impaired in carriers unable to develop sufficient antibody to the virus and in turn developing chronic hepatitis (27,28). With vaccination, above all recombinant vaccine, it is though that be administration of antigen in different quantities, different compositions of the virus and processing of the virus by antigen presenting cells may cause activation of the immune system. This hypothesis is supported by the findings of Akbar et al that MHC Class II CD 86 receptors on dendritic cells increased following vaccination (29).

In conclusion, hepatitis B vaccine is inexpensive and has few side-effects. Although its efficacy in hepatitis B infection has been demonstrated by a limited number of studies, it appears to be an effective treatment and may be an alternative to other treatment modalities. Further studies, in which the vaccine can be administered to patients not responding to other treatments for longer periods and with vaccines containing different antigens are needed.

REFERENCES

1. Szmunnes W, Stevens CE, Harley EJ, et al. Hepatitis B Vaccine: Demonstration of efficacy in a controlled clinical trial in a high risk population in the United States. N Eng J Med 1980;303: 833-41.
2. Alexander GJM, Brahm J, Fagan EA. Loss of HbsAg with interferon therapy in chronic hepatitis virus infection.

Lancet 1987; 2: 66-8.

3. Saracco G, Mazzella G, Rosina F. A controlled trial of human lymphoblastoid interferon in chronic hepatitis B in Italy. Hepatology 1989;10: 336-41.
4. Jules L, Dienstang MD, Eugene R et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. N Eng J Med 1999;341:1256-63.
5. Liaw YF, Leung NW, Chang TT et al. Effects of extended lamivudine therapy in Asian patients chronic hepatitis B. Asia hepatitis lamivudine study group. Gastroenterology 2000;119: 172-80.
6. Wong DKH, Cheung AM, Q'Rourke K, et al. Effect of alpha interferon treatment in patients with HBe antigen positive chronic hepatitis B. Ann Intern Med 1993; 119: 312-23.
7. Peters M, Vierling J, Grenswin ME, et al. Immunology of liver. Hepatology 1991;13: 977-94.
8. Guan R, Yap I, Wong L, et al. Evidence of viral replication in HBs Ag positive patients with hepatocelluler carcinoma : measurement of serum hepatitis B virus deoxyribonucleic acid. Ann Acad Med Singapore 1989; 88: 557-61.
9. LingaoAL, Domingo EO, Nishioka K. Hepatitis B virus profile of hepatocelluler carcinoma in the Philippines. Cancer 1981; 48: 1590-5.
10. Böcher WO, Herzog-Hauff S, Herr W, et al. Regulation of neutralizing antihepatitis B surface antibody response in vitro in HBs vaccine recipients and patients with acute or chronic hepatitis B virus infection. Clin Exp Immunol 1996; 105:52-8.
11. Eyıgun CP, Yılmaz S, Gul C, et al. A cooperative trial of two surface subunit recombinant hepatitis B vaccines vs a surface and PreS subunit vaccine for immunization of healthy adults. J Viral Hepat 1998; 5: 265-9.
12. Burke DS. Vaccine therapy for HIV, a historic view of the treatment of infectious diseases by active specific immunization with microbe-derived antigens. Vaccine 1993; 11: 883-91.
13. Stanberry LR, Bernstein DI, Burke RL, et al. Vaccination with herpes simplex virus glyco-proteins; protection against initial and reccurent genital herpes. J Infect Dis 1987; 155: 914-20.
14. Knodell RG, Ishak KG, Black WC. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; 1:431-5.
15. Pol S, Coullin I, Michel ML, et al. Immunotherapy of chronic hepatitis B by anti HBV vaccine. Acta Gastroenterol Belg 1998; 61: 28-33.
16. Kapke GF, Watson G, Sheffler S, et al. Comparison of the Chiron Quantiplex branched DNA (bDNA) assay and the Abbott Genostics solution hybridization assay for quantification of hepatitis B viral DNA. J. Viral Hepatitis 1997; 4: 67-75.
17. Coullin I, Pol S, Mancini M, et al. Specific Vaccine therapy in Chronic Hepatitis B: Induction of T Cell Proliferative Responses Specific for envelope Antigens. J Infect Dis 1999; 180: 15-26.
18. Pol S. Driss F, Michel M, et al. Specific vaccine therapy in chronic hepatitis B infection. Lancet 1994; 216: 342-8.
19. Wen Y-M, Wu X-H, Hu D-C, et al. Hepatitis B vaccine and anti HBs complex as approach for vaccine therapy. Lancet 1995; 345:1575-6.
20. Akbar SMF, Kazunori K, Kanimota K, et al. Placebo controlled trial of vaccination with hepatitis B virus surface antigen in Hepatitis B virus transgenic mice. J Hepatol 1997; 26: 131-7.
21. Löhr HF, Krung S, Herr W, et al. Quantitative and functional analysis of core specific T helper cell and CTL activities in acute and chronic hepatitis B. Liver 1998;18: 413-24.
22. Löhr HF, Weber W, Schlaak J, et al. Proliferative response CD4 T cells and hepatitis B virus clearance in chronic hepatitis with or without hepatitis B e-minus hepatitis B virus mutants. Hepatology 1995; 22: 61-8.
23. Imboden JB: T- Lymphocytes and Natural Killer Cells. In Medical Immunology. Stites DP, Abba I, Tristram T,Parslow G (Eds). Prentice-Hall International Inc.1997, p 130-45.
24. Mattilla PS, Pelkonen J, Makela O. Proportions of Ig G subclasses in antibodies formed during vaccination with hepatitis B surface antigen. Scand J Immunol 1986; 24: 699-704.
25. Böcher W, Herzog-Hauff S, Schlaak J, et al. Kinetics of hepatitis B surface antigen-specific immuner response in acute and chronic hepatitis B or after HBs vaccination: Stimulation of the in vitro antibody response by interferon gamma. Hepatology 1999; 29: 238-44.
26. Rahman F, Dahmen A, Herzog-Hauf S, et al. Cellular and humoral immune responses induced by intradermal or intramuscular vaccination with the major hepatitis B surface antigen. Hepatology 2000; 31: 521-7.
27. Egea E, Iglesias A, Salazar M, et al. The cellular basis for lack of antibody responses to hepatitis B vaccine in humans. J Exp Med 1991; 173: 531-8.
28. Kurose K, Akbar F, Yamamoto K, et al. Production of antibody to hepatitis B surface antigen (anti-HBs) by murine hepatitis B virus carriers: neonatal tolerence versus antigen presentation by dendritic cells. Immunology 1997; 92: 494-500.
29. Akbar SMF, Abe M, M Toshikazu, et al. Mechanism of activation of vaccine therapy in murine hepatitis B virus carriers: vaccine-induced activation of antigen presenting dendritic cells. J Hepatol 1999; 30: 755-64.