Govaresh/ Vol. 10, No. 4, Winter 2005; 227-232

Efficacy of Hepatitis B Virus Surface Antigen Vaccine as a Therapeutic Tool, in Inactive Hepatitis B Virus Carriers

Ghodrat Montazeri[1], Zahra Farzadi[2], Atossa Fazllolahi[2], Shifteh Abedian[2], Farhad Montazeri[2], Arezoo Estakhri[2], Negin Noori[2], Reza Malekzadeh[3]

[1] Associate Professor, Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran

[2] Research Fellow, Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran

[3] Professor, Digestive Disease Research Center, Tehran University of Medical Sciences

ABSTRACT

Background

Available drugs are not able to eradicate intracellular viral DNA in patients with hepatitis B virus (HBV) infections. HBsAg vaccine could induce immunity and subsequently eradicate Hepatitis B virus in proportions of these patients. Our aim was to evaluate the efficacy of HBsAg as a mode of therapy in inactive carriers.

Materials and Methods

Forty two consecutive patients of inactive carriers were enrolled. All patients underwent liver biopsies. The modified Ishak score of all cases were less than 4. Twenty microgram of recombinant HBsAg vaccine injected intradermally 3 times (at 0, one and 6 months). Biochemical and serological variables evaluated initially and 6 months after the last injection.

Results

The mean age was 39.6 ± 11.12. Male/female ratio was (67.4%). Two out of 42 cases lost their HBsAg (4.74%). The difference was significant comparing to one percent annual spontaneous HBsAg loss (p=0.014). In addition serum albumin level was significantly increased after vaccination (p=0.009). Rest of the biochemical and serological variables had no significant changes comparing pre and post vaccination.

Conclusions

Intradermal injection of HBV surface antigen vaccine could induce significant HBsAg loss. This mode of therapy is cheap, physiologic and without complication. However, the results of this study should be confirmed in further large controlled trial.

Keywords: Chronic hepatitis B, Vaccination, Inactive carrier

Govaresh/ Vol. 10, No. 4, Winter 2005; 227-232

BACKGROUND

HBV infection is a serious global health problem. Out of 2 billion people who have been infected with HBV worldwide, 300 million are suffering from chronic Hepatitis B.(1, 2), Chronic HBV infection is a major cause of end-stage liver diease. 25% of them will die prematurely from liver cirrhosis or hepatocellular carcinoma.(3)

FDA★ approved antiviral agents against HBV, either interferon or nucleoside analogues can

Corresponding author: Digestive Disease Research Center, Shariati Hospital, Kargar-e-Shomali Ave., Tehran, 14114, Iran.
Telefax: +98 21 88012992
E-mail: montazer@ams.ac.ir

★ Food and Drug Administration

relatively inhibit HBV replication. The rate of inhibition varies from 20-40%, depending on genotype, gender, race and chronicity.(4), Interferon is associated with lots of side effects. Emergence of drug resistant mutations and high relapse rate are two major draw backs for using nucleoside analogues. None of those therapeutic agents are able to eradicate intracellular HBV cccDNA.

Infected hepatocytes are eliminated by sensitized cytotoxic T-cells and those who have inadequate cellular immune response will remain chronically infected. Lack of efficacy and high cost of available drugs underline the importance of immune therapy for this disease. As a rule exogenous antigen is processed by HLA★ class II and stimulates humoral immunity. On the other hand, endogenous antigen is processed by HLA class I and stimulates cellular immunity. HBS antigen is an exception to this rule and is able to stimulate both pathways simultaneously.(5, 6, 7, 8)

It was shown that intradermal injection of HBS antigen in healthy volunteers could stimulate both pathway and the response was superior comprising to intramuscular injection.(9), In this work we aimed to evaluate the rate of HBS antigen loss in HBV inactive carrier patients by intradermal vaccination therapy.

MATERIALS AND METHODS

Patients and design

Forty two consecutive patients of naïve inactive carrier were selected. They had positive HBsAg, negative HBeAg and had normal transaminase 6 months prior to vaccination therapy. Liver biopsy was done for all cases. All biopsies were scored by modified HAI★★ scoring system by a single pathologist who had been unaware of patients' clinical condition.(10), Those cases that had score ≤3 were enrolled for vaccination therapy. Vaccine recipients received 3 intradermal immunizations with 1 ml HBV surface antigen vaccine (Hepavax-Gene, Green Cross Vaccine Corporation, South Korea) at month 0, 1, and 6 in Deltoid skin region. Each ml dose contain 20µg recombinant HBsAg adsorbed to approximately 0.5 mg of aluminum hydroxide. The vaccine formulation contains 0.01 w/v % thimersol added as a preservative. The protein was produced by culture of genetically engineered yeast cells which carry the relevant gene of HBsAg.

Six months after the last vaccination, patients were re-evaluated. Post vaccination re-evaluation consists of measuring transaminases and doing all HBV serology profile. Rate of HBsAg loss of vaccinated group compared to average annual HBS antigen loss of historical control from the medical literatures.

Statistical Analysis

Data were expressed as mean ±SD. One way analysis was used to compare rate of antigen loss in vaccination group versus historical control. Paired-sample t test was used to compare means. p-value<0.05 was used to indicate a significant difference.

RESULTS

Forty two consecutive patients were enrolled. Mean age was 39.6 ± 11.2 and 67.4% of the patients were male. Base line and 6 months post treatment biochemical and serological variable were compared. Two out of forty two patients had lost their HBsAg (4.74%). Comparing to an average historical annual HBsAg loss of 1%, the difference was statistically significant (p=0.014). One of these two patients had increased HBsAb>1:100 and the other one had HBsAb<1:5. There was no reversion of HBeAg and HBeAb. In addition serum level of albumin increased from 4.6 ± 0.38 to 4.87 ± 0.35 mg/dL. The difference was statistically significant (p=0.009). Rest of the biochemical variables had no significant changes comparing pre and post vaccination values (Table 1). No complication was observed.

★ Human Leukocyte Antigen
★★ Hepatitis Activity Index

Table 1. Levels of base line biochemical and serological variables were compared to post treatment counterparts.

Variables	Base line	6 months after vaccination	p value
ALT (IU/lit)	28.52 ±15.70	30.91 ± 24.03	0.483
AST (IU/lit)	25.70 ± 9.52	26.15 ± 14.60	0.815
Alkaline phosphatase (IU/lit)	171.45± 56.51	170.52 ± 50.03	0.906
Prothrombine time (seconds)	14.12 ± 1.14	14.02 ± 1.04	0.661
Albumin (gm/dl)	4.60 ± 0.54	4.87 ± 0.35	0.009
Bilirubine (mg/dl)	1. ± 0.38	1.06 ± 0.38	0.283
Platelet count (counts/mm^3)	230325± 63356	217325 ± 51372	0.081
HBsAg (+/-)	42/0	40/2	0.014

DISCUSSION

In this study we have shown that therapeutic vaccination by itradermal injection of hepatitis B surface antigen (HBsAg) was associated with significant HBsAg loss. In addition serum albumin level was significantly increased after vaccination. The reasons to use intradermal route and the possible mechanisms of actions were discussed.

The intradermal route has been evaluated in ill patients, such as those with renal failure in an attempt to improve immunogenecity. In that study sixty patients randomly divided into two groups. The group who had received 5 μg of intradermal vaccination had better response comparing to the group who had received standard intramuscular vaccination, 20 μg at 0, 1 and 6 months.(11), In another study intradermal vaccination 5 μg every 2 weeks for 8 doses could produce protective antibody response in 45.8% of non-responders in 24 patients of renal transplants who didn't produce anti HBsAb by conventional HBs immunization. In addition, one booster of intramuscular injection with 40 μg followed by this regimen increased to response rate to 100%.(12), In a prospective study of 425 care-workers given 2 μg HBsAg intradermally or 20 μg intramuscular route, the response was 81% comparing to 93% in intradermal group versus intramuscular group.(13), In another study by increasing the dose to 1/6 of intramuscular dose. The response appeared the same comparing to intramuscular route.(14), Intradermal injection of vaccine delivery is used in other viruses like influenza viruses and rabies with smaller doses. In one study 119 patients had received 40% of intramuscular dose. The antibody response was the same in both groups in age 18-60 years.(15, 16), The same effect was observed in rabies, too. Three doses of 0.1 ml intradermally have given lower response comparing to 3 doses of 0.5 ml intramuscular injection. Although intramuscular response was higher, but the difference was not statistically significant.(17), Intradermal route for HBsAg vaccination was evaluated in healthy people who were non-responders to conventional intramuscular injection. In one study, 5 μg HBsAg vaccine injected intradermally in 31 non-responders, every 2 weeks till development of delayed skin hypersensitivity. 94% developed protective antibody production. In addition, the same pattern of intradermal injection was used in 15 cases that have been accidentally exposed to specimen positive for HBeAg. After one year the protection was 100% in these cases.(18), HBs vaccine has been used as immunotherapy, and it was shown that it could control HBV replication. One study included 119 patients of chronic hepatitis B, 37 of them received no therapy. Forty six patients received preS2/S vaccine and 37 have received S vaccine 20 μg intradermally 5 times. HBV-DNA negativation was higher in vaccine group (16.3%),

than in control group (2.7%).(19)

The mechanisms of action of HBV vaccine therapy was studied in HBV- Transgenic mice. 27 of the 45 vaccine recipients HBV-Transgenic mice became negative for HBeAg and HBsAg in sera by injection 10 μg HBsAg emulsified in complete Freund's adjuvant. Responders had higher proliferation of specific lymphocyte; and higher capacity of dendritic cells to induce cellular and humoral immunity against HBsAg.(20), Th1 type stimulation happened by injecting HBsAg in patients with chronic hepatitis B.(21), HBV surface antigen vaccine could stimulate cellular immunity through cross-presentation. This phenomenon is an important function of dendritic cells in general, and intradermal Langerhans' cells in particular.(22-29), Dendritic cells are able to phagocytized exogenous antigen and load both on HLA-class II and HLA class I. Through this mechanism exogenous HBsAg will be able to produce both humoral and cellular immunity in healthy people and in some patients with chronic hepatitis B. During development, Langerhans' cells appear in the epidermis at six to seven weeks of gestation and are renewed continuously from a proliferative pool of myeloid, as well as multiplying in some within epidermis.(30, 31), In adult life they number 400-1000/mm^2.(32), These cells are regularly scattered through the epidermis and the stratified squamous epithelia of buccal mucosa. The dendritic Langerhans' cells, which make up 2-8% of epidermal cell population is a marrow derived macrophage expressing Ia cell surface antigen, FC and C3 cell surface receptor.(33), Interadermal Langerhans cells could be stimulated by an antigen and changed to active dendritic cells that can carry phagocytized Ag into lymph node and present it to the appropriate T and B cells. The presence of abundant dendritic cells in the epidermis could explain the higher response rate of intradermal injection as comparing to intramuscular injection.

Annual spontaneous rate of HBsAg are quite variable. One study from Taiwan reported annual rate of HBsAg as 0.5% in 984 patients with chronic hepatitis B by a mean follow-up 4 ± 2.5 years (1-12 years) and 0.8% in 1598 patients of inactive carriers with a mean follow-up 2.7 ± 1.4 years (1-10 years).(34), In another study, in which 420 children age 1-12 years have been followed up for a mean of 4.3 years (1-12 years), the annual spontaneous clearance rate was 0.6%.(35), The annual rate of HBsAg in adult carriers was reported to be 1% to 1.7% in two studies in the United States.(36, 37), In another longitudinal follow-up of 51 asymptomatic hepatitis B surface antigen positive, chronic patients for 1-15 years (median of 10 years), non had lost HBsAg.(38), Seventy eight patients who have been followed up for a mean of 5 years (1-12 years) had annual HBsAg loss of 1.9%. In this study, total patients who lost HBsAg were 5. Two of them belonged to 7 cases of acute hepatitis B in this group, and those cases who had not lost HBeAg (30% of total) was not part of calculation. By re-analysing the data, the actual loss of chronic carriers is going to be less than 1% annually.(39)

The main limitation of this study is that our work is an uncontrolled trial. We compared the rate of HBsAg loss with historical controls from other countries. However, a 4.74 % rate of HBsAg loss in our patient is promising.

CONCLUSION

In conclusion, we have shown that intrademal injection of HBsAg caused significant HBsAg loss. This form of therapy is cheap, easily available and without complication. Further works should be done on selecting proper antigenic epitope and adjuvant in order to increase therapeutic efficacy. However, the results of this study should be confirmed in further large controlled trials.

References

1. Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; 337: 1733-45. Review.
2. Kane M. Global programme for control of hepatitis B infection. *Vaccine* 1995; 13 Suppl 1:S47-9.
3. Mast EE, Aefen MJ. Epidemiology of viral hepatitis: An overview. *Sem Viral* 1993; 4: 273-83.

4. Liu CJ, Kao JH, Chen DS. Therapeutic implications of hepatitis B virus genotype. *Liver Int* 2005; 25:1097-107.
5. Yewdell JW, Bennink JR. Cell biology of antigen processing and presentation to major histocompatibility complex class I molecule-restricted T lymphocytes. *Adv Immunol* 1992; 52: 1-123.
6. Bohm W, Schirmbeck R, Elbe A, Melber K, Diminky D, Kraal G, *et al.* Exogenous hepatitis B surface antigen particles processed by dendritic cells or macrophages prime murine MHC class I-restricted cytotoxic T lymphocytes in vivo. *J Immunol* 1995; 155: 3313-21.
7. Schirmbeck R, Melber K, Mertens T, Reimann J. Antibody and cytotoxic T-cell responses to soluble hepatitis B virus (HBV) S antigen in mice: Implication for the pathogenesis of HBV-induced hepatitis. *J Virol* 1994; 68: 1418-25.
8. Bachmann MF, Lutz MB, Layton GT, Harris SJ, Fehr T, Rescigno M, *et al.* Dendritic cells process exogenous viral proteins and virus-like particles for class I presentation to CD8+ cytotoxic T lymphocytes. *Eur J Immunol* 1996; 26: 2595-600.
9. Rahman F, Dahmen A, Herzog-Hauff S, Bocher WO, Galle PR, Lohr HF. Cellular and humoral immune responses induced by intradermal or intramuscular vaccination with the major hepatitis B surface antigen. *Hepatology* 2000; 31: 521-7.
10. Ishak K, Baptista A, Bianchi L. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; 22: 696-9.
11. Chau KF, Cheng YL, Tsang DN, Choi KS, Wong KM, Chak WL, *et al.* Efficacy and side effects of inradermal hepatitis B vaccination in CAPD patients: A comparison with the intramuscular vaccination. *Am J Kidney Dis* 2004; 43: 910-17.
12. Choy BY, MalikPeiris JS, Chan TM, Lo SKF, Lui SL, Lai KN. Immunogenicity of intradermal hepatitis B vaccination in renal transplant recipients. *Am. J of Transplantation* 2002; 2: 965-9.
13. Coleman PJ, Shaw EF Jr, Serovich J, Hadler SC, Margolis HS. Intradermal hepatitis B vaccination in a large hospital employee population. *Vaccine* 1991; 9: 723-7.
14. Henderson EA, Louie TJ, Ramotar K, Ledgerwood D, Hope KM, Kennedy A. Comparison of higher dose intradermal hepatitis B vaccination to standard intramuscular vaccination of healthcare workers. *Infect Control Hosp Epidemiol* 2000; 21: 264-9.
15. Belshe RB, Newman FK, Cannon J, Duane C, Treanor J, Van Hoecke C, *et al.* Serum antibody responses after intradermal vaccination against Influenza. *N Engl J Med* 2004; 351: 2286-94.
16. La Montagne JR, Fauci AS. Intradermal Influenza vaccination-Can less be more? *N Engl J Med* 351, 22: 2330-2.
17. Redfield RR, Innis BR, Scott RM, Cannon HG, Bancroft WH. Clinical evaluation of low-dose intradermally administrered haepatitis B virus vaccine: A cost reduction strategy. *JAMA* 1985; 254: 3203-6.
18. Nagafuchi S, Kashiwagi S, Okada K, Anzai K, Nakamura M, Nishimura Y, *et al.* Reversal of nonresponders and postexposure prophylaxis by intradermal hepatitis B vaccination in Japanese medical personnel. *JAMA* 1991; 265: 2679-83.
19. Pol S, Nalpas B, Driss F, Michel ML, Tiollais P, Denis J, *et al.* Efficacy and limitations of a specific immunotherapy in chronic hepatitis B. *J of Hepatol* 2001; 34: 917-21.
20. Akbar SMF, Abe M, Masumoto T, Horiike N, Onji M. Mechanism of action of vaccine therapy in murine hepatitis B virus carriers: vaccine-induced activation of antigen presenting dendritic cells. *J of Hepatolog* 1999; 30: 755-64.
21. Couillin I, Pol S, Mancini M, Driss F, Brechot C, Tiollais P, *et al.* Specific vaccine therapy in chronic hepatitis B: Induction of T-cell proliferative responses specific for envelope antigens. *J of Infect Dis* 1999; 180: 15-26.
22. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, *et al.* Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; 18: 767-811.
23. Heath WR, Carbone FR. Cross-presentation, dendritic cells, tolerance and immunity. *Annu Rev Immunol* 2001; 19: 47-64.
24. Heath WR, Belz GT, Behrens GMN, Smith CM, Forehan SP, Parish IA, *et al.* Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol Rev* 2004; 199: 9-26.
25. Ktzler MA, Weiner DB. Developing DNA vaccines that call to dendritic cells. *J Clin Invest* 2004; 114: 1241-4.
26. Steinman RM, Pope M. Exploiting dendritic cells to improve vaccine efficacy. *J Clin Invest* 2002; 109: 1519-26.
27. Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. *Annu Rev Immunol* 2005; 23: 975-1028.
28. Rock KL, Goldberg AL. Degradation of cell proteins and the generation of MHC class I-presented peptides. *Annu Rev Immunol* 1999; 17: 739-79.
29. Szabo SJ, Sullivan BM, Peng SL, Glimcher LH. Molecular mechanisms regulating TH1 immune responses. *Annu Rev Immunol* 2003; 21: 713-58.
30. Foster CA, Holbrook KA, Farr AG. Ontogeny of Langerhans' cells in human embryonic fetal skin: Expression of HLA-DR and OKT-6 determinants. *J Invest Dermatol* 1986; 86: 240-43.
32. Czermelewski JM, Demarchez M. Future evidence for the self reproducing capacity of langerhans cells in human skin. *J Invest Dermatol* 1987; 88: 17-20.
31. Katz SI, Tamaki K, Sachs DH. Epidermal Langerhans' cells are derived from cells which originate in bone marrow. *Nature* 1979; 282: 324-6.
33. Diazy LA, Provost TT. Dermatologic disease. In: Sides DP, Stobo JD, *et al.,* editors. Basic and clinical immunology. 6th ed. Lange Medical Books: 1987. p. 516-33.
34. Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidents, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: A prospective study. *Hepatology* 1991; 13: 627-31.
35. Hsu HY, Chang MH, Lee CY, Chen JS, Hsu HC,Chen DS. Spontaneous loss of HBsAg in children with chronic Hepatitis B virus infection. *Hepatology* 1992; 15: 382-6.

36. Sampliner RE, Hamilton FA, Iser OA, Tabor E, Boitnott J. The liver histology and frequency of clearance of the hepatitis B surface antigen (HBsAg) in chronic carriers. *Am J Med Sci* 1979; 227: 17-22.
37. Alward WLM, McMahon BJ, Hall DB, Heyward WL, Francis DP, Bender TR. The long-term serological course of asymptmatic hepatitis B virus carriers and the development of primary hepatocellular carcinoma. *J Infect Dis* 1985; 151: 604-9.
38. Lok ASF, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive Chinese children. *Hepatology* 1988; 8: 1130-3.
39. Bortolotti F, Cadrobbi P, Crivellaro C, Guido M, Rugge M, Noventa F, *et al.* Long-term outcome of chronic type B hepatitis in patient who acquire hepatitis B virus infection in childhood. *Gastroenterology* 1990; 99: 805-10.