Papers

Effect of timing of hepatitis B vaccine doses on response to vaccine in Yucpa Indians

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In a large hepatitis B prevention programme, hepatitis B vaccine was given in standard doses to >1000 susceptible Yucpa Indians between 1983 and 1985. Thirteen months after the programme began, 373 vaccine recipients were tested using commercial radioimmunoassay to titre antibody response to the vaccine. Because of logistic difficulties, only 32% had received vaccine by the recommended schedule (second and third doses at one and six months after the first, respectively). The second and third doses were received early by 4 and 31%, respectively, and 27 and 16% received these doses later than intended. Overall response to vaccine was excellent: 98% of vaccinees developed anti-HBs >10 mIU (geometric mean titre 688 mIU). Multivariate analysis showed that the response to vaccination was inversely related to the age of the vaccinee and directly related to the timing of the third vaccine dose. In particular, those receiving the third vaccine dose late (>7 months after the first dose) developed antibody titres two-fold higher than those receiving the third dose on schedule (p<0.01). The response to vaccination was not significantly related to the timing of the second dose. A satisfactory response was obtained with various schedules of dose timing, including early second and third doses, late second and third doses and late second but normal third doses. These findings suggest that the response to hepatitis B vaccine is not highly dependent on timing of vaccine doses and that modest alterations in timing of doses, such as those necessary to integrate hepatitis B vaccine with other childhood vaccines, do not affect the excellent response to this vaccine.

Keywords: Hepatitis B vaccine; dose schedule

Introduction

Vaccines currently available are highly immunogenic and effective in preventing hepatitis B virus (HBV) infection in children and adults when given as recommended[1–8]. In particular, studies conducted worldwide have shown that the plasma-derived hepatitis B vaccine licensed in the US is highly effective when given as a three-dose series, in which the second and third doses are given one and six months, respectively, after the first dose.

Nevertheless, the vaccine schedules devised for optimal vaccine use in the developed world may not be practical for countries in the developing world. Difficulties in reaching potential vaccinees may result in a delay in receiving some doses. More importantly, integration of hepatitis B vaccine as a universal immunogen of early childhood, a probable necessity for the global prevention of hepatitis B, is not easily accomplished with rigid specifications on the timing of the vaccine doses[9]. Flexibility in timing of doses would facilitate such integration and minimize the number of visits required by children to receive all vaccines. Although the vaccine manufacturer in the US initially conducted experiments varying the timing of vaccine doses and several other groups have since shown a modest effect on vaccine response when the timing of the second and/or third vaccine doses are varied, little practical information exists regarding the impact of varying the timing of vaccination in a field setting (A.E. McLean, personal communication)[10,11].

In 1983, following studies that demonstrated high endemicity of HBV infection in the Yucpa Indians in western Venezuela, a programme of HB vaccination of susceptible persons in this population was initiated[12]. Although the study was designed to deliver three doses by the recommended schedule, difficulties in reaching these persons led to wide variation in delivery of the vaccine doses. Subsequent serological testing has, however, shown an excellent response to the vaccine and provides a practical measure of the impact of varying the vaccination schedule from that which is normally used.

Materials and methods

In April 1983, a programme to vaccinate all Yucpa Indians against hepatitis B was begun in western Venezuela. Following approval of the programme by Venezuela health authorities and tribal and village leaders, all adults and children >3 years old were bled to screen for prior HBV infection. Beginning in June 1983, all

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susceptible adults and older children and all children aged ≤3 years were offered plasma-derived hepatitis B vaccine (Heptavax, Merck, Sharp & Dohme). Adults and children >9 years of age were given three 20 μg doses of vaccine and children <10 years given three 10 μg doses of vaccine according to the manufacturer's recommendation (second dose one month after the first, third dose six months after the first). All vaccine was given in the deltoid muscle, except in some infants in whom it was given in the anterolateral thigh muscle.

During the first four months of the study, 1037 persons received the first dose and, over the ensuing year, 895 of these (88%) completed the three-dose series. Nevertheless, due to logistic difficulties in regularly visiting villages and to the itinerant nature of this population, the timing of vaccine doses frequently deviated from the recommended schedule. The timing of the second dose varied from as few as two weeks to as long as five months after the first, while the third dose was given between three and 12 months after the first.

To assess response to the vaccine, investigators obtained serum specimens from 416 randomly selected vaccinees between 9 and 16 months after receipt of the first vaccine dose. All serum specimens (both prevaccination and postvaccination samples) were initially tested for antibodies to hepatitis B surface antigen (anti-HBs) and hepatitis B core antigen (anti-HBc) by radioimmunoassay (Ausab and Corab, Abbott Laboratories). In postvaccination testing, all specimens positive for anti-HBc were confirmed by repeat testing and tested for hepatitis B surface antigen (HBsAg) by radioimmunoassay (Ausria II; Abbott Laboratories). For all vaccinees with negative anti-HBc on postvaccination specimens, anti-HBs titres were determined in International units (mIU ml^{-1}) by a standard procedure[11].

Analysis

The response to the vaccine was examined in all vaccinees remaining uninfected (anti-HBc negative) at postvaccination follow-up. The presence of a protective anti-HBs response (> 10 mIU ml^{-1}) and the geometric mean titres (GMT) of anti-HBs were examined by univariate analysis to assess variation of the response with demographic factors (age, sex, village of origin) and vaccination parameters (timing of the second and third vaccine doses, timing of postvaccination testing). In the univariate analysis, the frequencies of response were compared using Fisher's exact test and the logarithms of GMTs compared by Student's *t*-test.

Variables that were significant predictors of response were then entered into multiple linear regression using the logarithm of the titre of anti-HBs as the dependent variable, age and vaccination parameters as continuous independent variables, and sex and village groups as non-ordered categorical variables, using the Statistical Analysis System[13]. Persons with no detectable antibody were included in GMT calculations using a logarithm of titre equal to zero.

Results

Among the 416 Yucpa Indians tested after completing vaccination, 195 were infants and young children under four years of age who had not been screened before vaccination. The remainder were older children and adults who were screened and found to be negative for both anti-HBs and anti-HBc before vaccination. Among the infants and young children, 34 were found postvaccination to be positive for anti-HBc (seven were also positive for HBsAg); these children were most probably infected prior to vaccination. Nine older children or adults were anti-HBc-positive on follow-up; these persons acquired infection while receiving the vaccine series. Both groups were eliminated from the analysis of response to the vaccine.

Among the 373 Yucpa who completed vaccination and remained negative for anti-HBc, the response to the vaccine was excellent. Four (1.1%) persons did not respond to the vaccine series, and four others (1.1%) developed low levels of antibody (<10 mIU ml^{-1}). Among the 98% who did respond to vaccination, anti-HBs levels after the third dose ranged from 10 to 195 400 mIU ml^{-1}; the GMT anti-HBs response for the entire group was 688 mIU.

Univariate analysis showed some variation of response with age, village group and timing of receipt of the second and third vaccine doses (*Table 1*). Only among vaccinees >20 years of age was the likelihood of non-response to the vaccine significantly increased above that of other vaccinees; for the other variables, differences in overall response rates were not significant. The GMT of anti-HBs, however, a more sensitive measure of response to the vaccine, showed more extensive variations. The response did not differ among persons <20 years of age, but in such persons was about threefold higher than in adults >20 years of age ($p=0.01$). Sex had no impact on the vaccine response in the univariate analysis; however, village location did have some effect, with persons living in northern Yucpa villages (NBA) having a significantly lower response than those living in the central village (Tukuko).

The antibody titre increased steadily as the interval between the first and third vaccine doses increased; the response was reduced by 30% in persons who received the third dose >1 month early ($p=0.05$) and was enhanced twofold in those who received the third dose more than one month late ($p<0.01$) (*Table 1*). In those

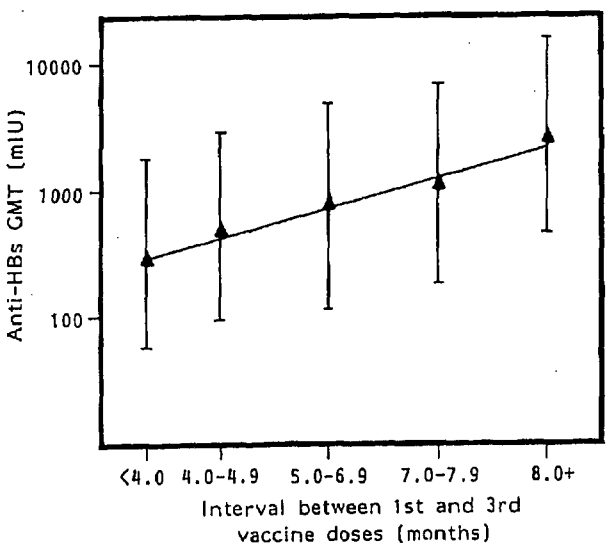

Figure 1 Effect of varying the timing of the third vaccine dose on the response to hepatitis B vaccine in Yucpa Indians. Bars indicate s.d.

Table 1 Univariate analysis of antibody response

Variable	No. tested	No. (%) ≥10 mIU	Geometric mean titre (mIU ml^{-1})	Significance
Age <20 yrs	331	328 (99.1)	767	–[a]
>20 yrs	33	28 (84.8)	239	0.01
Unknown	9	9 (100)	621	
Sex M	191	186 (97.4)	706	–[a]
F	181	178 (98.4)	668	NS
Village TUK	149	146 (98.0)	998	–[a]
MTN	35	35 (100)	723	NS
SBA	52	51 (98.1)	644	NS
NBA	137	133 (97.1)	467	<0.002
Dose 2 (months after dose 1)				
0.4–0.6	16	16 (100)	493	NS
0.7–1.4	252	247 (98.0)	659	–[a]
>1.5	105	102 (97.1)	805	NS
Dose 3 (months after dose 1)				
<5.0	136	132 (97.1)	461	0.05
5.0–6.9	163	159 (97.5)	685	–[a]
>7.0	74	74 (100)	1435	<0.01
Test (months after dose 1)				
<13.0	253	249 (98.4)	734	–[a]
>13.0	120	116 (96.7)	603	NS
Interval dose 2 to dose 3 (months)				
<4.0	197	191 (97.0)	536	0.10
4.0–5.9	131	129 (98.5)	752	–[a]
>6.0	45	45 (100)	1606	0.02
Interval dose 3 to test (months)				
<5.0	75	75 (100)	1349	<0.002
5.0–7.9	214	209 (97.7)	632	–[a]
>8.0	84	81 (96.4)	471	NS

[a] Reference group in each category

Table 2 Multiple linear regression analysis of the effect of various factors on the response to hepatitis B vaccine

Factor	Slope of log anti-HB_s titre (s.d.)	Significance
Age (yr)	−0.018 (0.005)	0.0007
Interval between first and third vaccine doses (months)	0.146 (0.055)	0.0085
Village group–north base	−0.509 (0.188)	0.0071

R^2 for model=0.107

Table 3 Summary of the effect of varying HB vaccine schedule on the response to the vaccine

Vaccine schedule		No.	Anti-HBs	
Dose 2[a]	Dose 3[b]	vaccinated[c]	GMT (mIU)	Significance[d]
Early	Early	11	242	0.05
On time	Early	89	411	0.02
Late	On time	39	418	0.10
On time	On time	118	743	–
Late	Early	36	748	NS
On time	Late	45	1222	0.10
Very late	Late	17	1622	0.05
Late	Late	13	2825	0.01

[a] Dose 2: early if <21 days after dose 1; late if >45 and <120 days after dose 1; very late if >120 days after dose 1
[b] Dose 3: early if <150 days after dose 1; late if >210 days after dose 1
[c] Five observations missing because did not fit into any schedule
[d] Compared with all doses on time

who received the third dose at least two months late, the GMT anti-HBs response was 2300 mIU ml^{-1}, threefold higher than in those who received this dose on schedule (*Figure 1*). The response to the vaccine did not vary significantly with the intervals between receiving the first dose of vaccine and either receiving the second dose of vaccine or having postvaccination testing.

A trend for increasing response with increasing interval between the first and second dose was noted, however, and the large number of persons who received the second dose late (>6 weeks after the first) were found to have a slightly better response than those receiving it on time (p=NS).

To clarify further the effect of timing of vaccine doses, the effect on the response to the vaccine of variation in the intervals between the second and third vaccine doses and between the third dose and postvaccination testing was examined. Increasing the interval between the second and third doses was positively correlated with vaccine response (p=0.02), while decreasing the interval between the third dose and testing was associated with a better vaccine response (p<0.002).

When all the above factors were entered into a multiple linear regression model, three factors – age, time interval between giving the first and third doses of vaccine, and living in the northern Yucpa villages – independently predicted the response to the vaccine (*Table 2*). The overall parameters of response did not differ markedly from those in the univariate analysis. The

overall correlation coefficient for the model was 0.107, indicating that these factors accounted for only a small proportion of the actual variation in the vaccine response in this population.

Finally, the response to the vaccine among persons grouped according to the timing of both the second and third vaccine doses was examined (*Table 3*). The overall risk of non-response to the vaccine did not differ among any of the groups, but the GMT antibody levels after vaccination was found to vary modestly. Persons who received both the second and third doses early had a 68% lower response, and those who received only the third dose early (but the second dose on time) had a 44% lower response than those who received all doses on time. In contrast, those who received both the second and third doses late had improved responses to the vaccine.

In addition, those who received the third dose very late (>8 months after the first) also had a significantly better response than those who received all doses on time. Nevertheless, the variation in geometric mean titres was not generally large, indicating that altered vaccination schedules had only a modest impact on response to vaccine.

Discussion

These studies of hepatitis B vaccination in the Yucpa Indians provide practical information on the use and expected impact of hepatitis B vaccines in less developed countries. First, they show an overall excellent response to the vaccine in children and adults given usual doses of vaccine under difficult field conditions. The proportion of persons developing an adequate protective response was comparable to studies in adults and children in controlled studies in the developed world. The GMTs of antibodies reached in this predominantly young population appeared somewhat lower than those found in children in other studies using this vaccine[14] (A.E. McLean, personal communication). Given that the vaccinees were tested ≈6 months after the final dose, however, the response was comparable to that usually seen in adults in the US and Europe[1,3,11,15].

Studies with a different vaccine in children in Senegal suggest that the response in children in less developed countries may be lower than that observed in children in developed countries[16]. Our findings also suggest this, but will await controlled vaccine trials.

Our study found several factors that influenced the response to the vaccine. Age >20 years was significantly associated with a decreased response to the vaccine. Other studies have shown a decreasing response to vaccine with age, which becomes most apparent after age 50[8,16]. We found little variation of response under age 20, as reported using the same vaccine in Alaskan Eskimos[8].

We also found that the response to the vaccine in one group of villages was significantly lower than in the central village. The reasons for this are not certain; no gross nutritional, ethnic or socioeconomic differences in the two populations were apparent. One difference is that the villages in the variant group are distant from the central vaccine storage facility and, thus, vaccine must be transported further (and for a longer duration) than to other villages. Handling of the vaccine may affect the response to the vaccine, due to inadvertent freezing or possibly to exposure to high ambient temperatures and, thus, may explain the small variation seen in our study[18,19].

Most importantly, we were able to quantify the effect of various vaccine dose schedules on the response to the vaccine. Although this was not a clinical trial, but a natural experiment dictated by field conditions, it provides useful insights into this problem. Clearly, variation of timing of the third vaccine dose had the most important effect on the antibody response measured at a fixed interval after the vaccinees received the first dose. This effect was linear, with an early third dose resulting in a lower than anticipated response, and a delayed third dose resulting in an increasing response. Our results confirm the findings of others on the positive effect of delaying the third vaccine dose and expands this to show the linear nature of this effect over a wide range of intervals[10,11].

Interestingly, varying the timing of the second dose had less impact on the response, although a trend similar to that found for the third dose was observed. The few persons who received the second dose early had a significantly lower response to the vaccine when the third dose was also given early and it would be prudent to avoid giving this dose too early. On the other hand, delay of the second dose for several months occurred in >100 vaccinees and had a positive effect on the response to the vaccine. Our findings contrast with those of Hollinger, who found a somewhat decreased response to a delayed second vaccine dose[11]. The reasons for this are uncertain, but it should be noted that in both studies differences were of borderline statistical significance and relatively small with respect to natural variation in response to the vaccine.

These findings have two practical implications. First, modest adjustment of the dose schedule of this vaccine, as may be necessary to integrate this into routine childhood immunization, can be expected to have a minimal impact upon the response to the vaccine. Delays in timing of the second dose to coincide with the bimonthly schedule for diphtheria–pertussis–tetanus vaccine, or of the third dose to coincide with measles vaccination, may actually improve the overall response at a small cost of briefly delaying complete protection. Hence, it appears this vaccine does have sufficient flexibility to be integrated easily into childhood immunization schedules. Nevertheless, controlled studies of vaccine immunogenicity and, possibly, efficacy by different schedules should be undertaken before adopting major changes in vaccine scheduling.

Second, minor delays experienced in giving the second and third vaccine doses in vaccination programmes are unlikely to have a major negative impact on vaccine response. Thus, delays of the second dose for several months, and of the third dose for up to six months, will not necessitate restarting the vaccine series or confirmation of the final response to the vaccine. With larger deviations from these schedules, it remains prudent to either complete the series and confirm the response with postvaccination testing, or to restart the whole vaccine series.

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References

1 Szmuness, W., Stevens, C.E., Zang, E.A., Harley, E.J. and Kellner, A. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. *Hepatology* 1981, **1**, 377

2 Francis, D.P., Hadler, S.C., Thompson, S.E. *et al.* The prevention of hepatitis B with vaccine: report of the Centers for Disease Control multi-center efficacy trial among homosexual men. *Ann. Intern. Med.* 1982, **97**, 362

3 Szmuness, W., Stevens, C.E., Harley, E.J. *et al.* Hepatitis B vaccine in medical staff of hemodialysis units: efficacy and subtype cross-protection. *N. Engl. J. Med.* 1982, **307**, 1481

4 Maupas, P., Goudeau, A., Coursaget, P., Drucker, F., Barin, F. and Andre, M. Immunization against hepatitis B in man: a pilot study of two years' duration. In: *Viral Hepatitis: a Contemporary Assessment of Etiology, Epidemiology, Pathogenesis and Prevention* (Eds Vyas, G.N., Cohen, S.N. and Schmid, R.) Franklin Institute Press, Philadelphia, 1978, pp. 539–556

5 Crosnier, J., Jungers, P., Courouce, A.-M. *et al.* Randomised placebo-controlled trial of hepatitis B surface antigen vaccine in French haemodialysis units: I. Medical staff. *Lancet* 1981, **I**, 455

6 Coutinho, R.A., Lelie, N., Albrecht-van Lent, P. *et al.* Efficacy of a heat inactivated hepatitis B vaccine in male homosexuals: outcome of a placebo controlled double blind trial. *Br. Med. J.* 1983, **286**, 1305

7 Dienstag, J.L., Werner, B.G., Polk, B.F. *et al.* Hepatitis B vaccine in health care personnel: safety, immunogenicity, and indicators of efficacy. *Ann. Intern. Med.* 1984, **101**, 34

8 Heyward, W.L., Bender, T.R., McMahon, B.J. *et al.* The control of hepatitis B virus infection with vaccine in Yupik Eskimos: Demonstration of safety, immunogenicity, and efficacy under field conditions. *Am. J. Epidemiol.* 1985, **121**, 914

9 Maynard, J.E., Kane, M.A., Alter, M.J. and Hadler, S.C. Control of hepatitis B by immunization: global perspectives. In: *Viral Hepatitis and Liver Disease* (Ed. Zuckerman, A.J.) Alan R. Liss Inc., New York, 1988, pp. 967–969

10 Jilg, W., Schmidt, M. and Deinhardt, F. Immune responses to late booster doses of hepatitis B vaccine. *J. Med. Virol.* 1985, **17**, 249

11 Hollinger, F.B., Adam, E. Heiberg, D. and Melnick, J.L. Response to hepatitis B vaccine in a young adult population. In: *Viral Hepatitis: 1981 International Symposium* (Eds Szmuness, W., Alter, H.J. and Maynard, J.E.) Franklin Institute Press, Philadelphia, 1982, pp. 451–466

12 Hadler, S.C., Monzon, M., Ponzetto, A. *et al.* Delta virus infection and severe hepatitis. An epidemic in the Yucpa Indians of Venezuela. *Ann. Intern. Med.* 1984, **100**, 339

13 *SAS/Graph Users Guide*, Version 5, SAS Institute Inc., Cary, NC, 1985

14 Matsaniotis, N., Kattamis, C., Dionyssopoulou, E. and Laskari, S. Immunogenicity of hepatitis B vaccine in normal children. *Vaccine* 1985, **3**, 297

15 Redfield, R.R., Innes, B.L., Scott, R.N., Cannon, H.G. and Bancroft, W.H. Clinical evaluation of low dose intradermally administered hepatitis B virus vaccine. *J. Am. Med. Assoc.* 1985, **254**, 3203

16 Yvonnet, B., Coursaget, P., Chotard, J. *et al.* Hepatitis B vaccine effects in infants from an endemic area: long-term anti-HBs persistence and revaccination. *J. Med. Virol.*, in press

17 Denis, F., Mounier, M., Hessel, L. *et al.* Hepatitis B vaccination in the elderly. *J. Infect. Dis.* 1984, **149**, 1019

18 McLean, A.A. and Shaw, R. Jr. Hepatitis B virus vaccine. *Ann. Intern. Med.* 1982, **97**, 451

19 Hanna, J.N. Poor response to hepatitis B vaccine administered to aboriginal infants in central Australia. *Med. J. Aust.* 1987, **147**, 504.