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Study design for a hepatitis B vaccine trial

(constant infection risk/renal dialysis patients/antibody against surface antigen of hepatitis B)

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ABSTRACT **A short-time trial of small sample size for an evaluation of the hepatitis B vaccine is proposed and designed. The vaccine is based on the premise that antibody to the surface antigen of the hepatitis B virus is protective against viral infection. This premise is verified by using the presence of the surface antigen as the marker of infection and comparing infection rates in renal dialysis patients who had naturally acquired antibody to patients without antibody. Patients with antibody have an extremely low risk of infection. The probability of remaining uninfected decreases at an exponential rate for patients without antibody, implying a constant risk of infection at any point in time. The study design described makes use of this time independence and the observed infection rates to formulate a clinical trial which can be accomplished with a relatively small number of patients. This design might be useful if, in preliminary studies, it is shown that the vaccine produces antibody in the patients and that protection against hepatitis B virus would be beneficial to the patients.**

Studies on the transmission of hepatitis B virus and its surface antigen (HB_sAg, Australia antigen) provide a large and well-documented data base. The difficulty in developing a proper mathematical model of transmission is due to the fact that the virus has unusual properties which are not completely understood (1, 2). Data on the prevalence and incidence of infection are, in many cases, the only evidence one can use to estimate the risk of morbidity.

Therefore, the majority of studies undertaken to characterize transmission have been population screenings which have revealed many interacting factors, including genetic, age, sex, maternal, and environmental effects. The response to infection with the virus can be one or more of a number of possibilities, including development of antibody against the surface antigen (anti-HB_s) and/or the core antigen (anti-HB_c), mild or severe illness, and transient or persistent HB_sAg (and presumably hepatitis B virus) in the infected individual's blood. Furthermore, there is great variability in the relative importance of the various factors in transmission and in the form of the response from one geographic area to another.

A technique has been developed for the preparation of a vaccine against hepatitis B (3). Animal studies (in chimpanzees) have been encouraging, and clinical trials are now being planned (4–6). However, the variety of factors affecting transmission dictates that great care must be taken in selecting a population for testing the vaccine and in designing the field trial.

An interesting population for investigation of the efficacy of a vaccine would be one where there is great risk of infection that can mainly be attributed to environmental circumstances. Further, it would be desirable for infected individuals to develop and retain the antigen for sufficient time to assure its detection. That is, the detection of HB_sAg would serve as the marker of infection, and the individuals in the test population would not develop anti-HB_s without first producing detectable HB_sAg. In such a population one could test whether the vaccine prevented infection with a minimum number of people in a short period of time.

We have monitored a community-based renal dialysis clinic which meets these criteria. The data obtained from the patients at the clinic include sufficient serial samples to formulate a descriptive model of transmission. Such a model was presented by Blumberg *et al.* (7) and is summarized here. From these data, it is apparent that anti-HB_s is protective against development of HB_sAg. This important conclusion, along with the mathematical features of the model, are extended in this paper to the design of a vaccine study.

Two potential designs are presented. One design follows a controlled clinical trial format where one specifies a significance level and a length of time desired for the study in order to obtain an estimate of sample size that must be tested. The second design is based on the use of historical controls, which, in this instance, would represent one of the rare occasions where that technique appears to be valid and appropriate. That is, the data analyses in this paper demonstrate that the risk of infection is constant over time and is independent of the length of time a patient has been at the clinic.

This paper emphasizes the phase of study in which patients at risk are vaccinated and subsequently exposed. Additional evidence is necessary to guarantee the advisability of vaccinating renal dialysis patients. Further animal studies will also be required to assure the safety of vaccine. The first human studies will probably be in volunteers who have a low probability of developing hepatitis in order to demonstrate that the vaccine will not produce hepatitis or other diseases. Should these animal and volunteer studies prove successful and should the vaccine appear to be beneficial to the patients, then the trial discussed in this paper would be appropriate. It is possible that the renal dialysis patients who have not already made anti-HB_s in response to natural infection are immunologically incapable of making antibody with a vaccine stimulation. A negative result from this study would not distinguish vaccine failure from immune incompetence. In this sense, then, the study design proposed here represents a more severe test of the vaccine than would ordinarily be required. However, the great advantage of this study, as will be demonstrated, is that it can be accomplished with an unusually small number of subjects. Further, if it is successful, this study would provide a firm basis for larger trials.

MATERIALS AND METHODS

The Delaware Valley Artificial Kidney Clinic is an outpatient facility operated separately from a hospital. Since its inception in November 1970, we have obtained monthly

Abbreviations: HB_sAg, surface antigen of hepatitis B; anti-HB_s, antibody against HB_sAg.

Table 1. Probability of not developing HB_sAg for patients who *did not* have anti-HB_s or HB_sAg at time of admission

1 Months at risk	2 Total patients	3 Convert to HB_sAg	4 Convert to anti-HB_s	5 Withdrawn from risk $HB_sAg(-)$	6 Remain $HB_sAg(-)$	7 Probability of not developing HB_sAg Observed	8 Predicted	9* SD
1	159	0	0	25	134	—	—	—
2	134	6	2	13	113	0.953	0.973	0.019
3	113	5	1	10	97	0.908	0.892	0.026
4	97	4	0	10	83	0.869	0.817	0.032
5	83	9	2	6	66	0.770	0.749	0.042
6	66	5	2	6	53	0.708	0.687	0.047
7	53	8	1	1	43	0.599	0.629	0.053
8	43	3	1	4	35	0.554	0.577	0.055
9	35	4	1	2	28	0.488	0.529	0.058
10	28	1	1	1	25	0.470	0.485	0.058
11	25	2	0	1	22	0.432	0.444	0.060
12	22	2	1	1	18	0.391	0.407	0.061
13	18	0	0	1	17	—	—	—
14	17	1	0	1	15	—	—	—
15	15	1	0	2	12	0.343	0.313	0.062
16	12	2	0	0	10	—	—	—
17	10	2	0	1	7	—	—	—
18	7	0	0	1	6	0.217	0.241	0.063
19	6	0	0	0	6	—	—	—
20	6	1	0	0	5	—	—	—
21	5	0	0	0	5	0.183	0.186	0.062
22	5	0	0	1	4	—	—	—
23	4	0	0	3	1	—	—	—
24	1	0	0	0	1	0.130	0.143	0.061
25+	1							

* Standard deviation of the observed value.

blood specimens from the patients and staff members. Patients leave the clinic for a variety of reasons, including death, renal transplantation, and transfer to other dialysis clinics. The results reported here are based upon 223 patients who have had at least one blood specimen tested for both HB_sAg and anti-HB_s before November, 1974. (Since the analysis in this paper depends upon estimating the rate of development of HB_sAg, 24 patients who entered the clinic with HB_sAg were excluded and not counted among the 223 patients considered.)

The blood specimens were tested at the laboratories of The Institute for Cancer Research for serum glutamic-pyruvic transaminase (alanine aminotransferase; EC 2.6.1.2) by Henry's modification of Karmen's method (8), for HB_sAg by immunodiffusion (9) and by counter-electrophoresis (10), and for anti-HB_s by passive hemagglutination (11). When the initial studies were done, radioimmunoassay for HB_sAg was not generally available. For consistency, this report includes only the results obtained by the less sensitive methods. Anti-HB_s tests were performed with both HB_sAg/ad and HB_sAg/ay coated red cells. A titer of 1:10 or greater was scored as positive.

RESULTS

There were 159 patients who had no detectable HB_sAg or anti-HB_s in their blood at the time of admission. The rate that these patients develop HB_sAg is given in Table 1 and shown in Fig. 1. Patients are considered withdrawn (Table 1, column 5) at a time of t months if the patient leaves the clinic after t months or has been at the clinic for a maximum of t months. Patients who develop anti-HB_s (column 4) were considered no longer at risk for developing HB_sAg.

Inspection of the data suggested an exponential rate of infection. A model for the probability of remaining uninfected of the form

$$Pr = 1 - \{1 - \exp[\alpha - \lambda(t - 1)]\}^n \qquad t = 2,3,\ldots,24$$

was considered. That is, this mathematical formula describes the shape of the curve obtained from the observed infection rate given in Table 1 (column 7). In this model the time (t) is measured in months, and $1/\lambda$ is the mean time until the occurrence of one event with n such events required for conversion. The parameter α is a scaling value introduced since there were no converters during the first month. The best fit values were found to be $n = 1$, $\lambda = 0.087$, and $\alpha = 0.06$ by using the Nelder-Mead algorithm (nonlinear least squares) with the observed probabilities weighted inverse to their

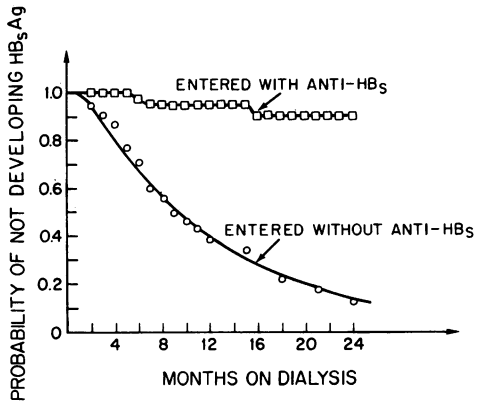

FIG. 1. Probability of not developing HB_sAg for patients admitted with and without anti-HB_s.

Table 2. Probability of not developing HB_sAg for patients who *did* have anti-HB_s at time of admission

1 Months at risk	2 Total patients	3 Convert to HB_sAg	4 Withdrawn from risk $HB_sAg(-)$	5 Remain $HB_sAg(-)$	6 Probability of not developing HB_sAg
1	64	—	8	56	1.000
2	56	0	4	52	1.000
3	52	0	1	51	1.000
4	51	0	6	45	1.000
5	45	0	2	43	1.000
6	43	1	3	39	0.976
7	39	1	1	37	0.951
8	37	0	1	36	0.951
9	36	0	5	31	0.951
10	31	0	1	30	0.951
11	30	0	0	30	0.951
12	30	0	1	29	0.951
13	29	0	0	29	—
14	29	0	0	29	—
15	29	0	4	25	0.951
16	25	1	5	19	—
17	19	0	2	17	—
18	17	0	1	16	0.908
19	16	0	2	14	—
20	14	0	1	13	—
21	13	0	0	13	0.908
22	13	0	2	11	—
23	11	0	1	10	—
24	10	0	4	6	0.908
25+	6				

standard deviation (12). The residual sum of squares for this model is 6.20 and is not significant since it is less than 22.4, the 5% point of the chi-square distribution with 13 degrees of freedom. The predictive capability of the model can be seen by comparing the predicted values (column 8) to the observed values (column 7) in Table 1.

The model estimates that at 9 months there is approximately a 50% chance of remaining uninfected, while at 24 months the probability drops to 15% (Fig. 1). Of the 64 patients who entered with anti-HB_s, only three subsequently developed HB_sAg (Table 2 and Fig. 1). Only 12 of the 159 patients entering without anti-HB_s or HB_sAg made anti-HB_s before having HB_sAg, and none of these subsequently developed HB_sAg. Furthermore, London *et al.* (W. T. London, J. S. Drew, E. D. Lustbader, and B. S. Blumberg, in preparation) have shown that those renal dialysis patients who convert to HB_sAg have approximately a 60% chance of remaining a carrier indefinitely and that the carriers in general have mildly or moderately elevated serum activities of glutamic-pyruvic transaminase.

These results can be summarized as follows:

(a) Patients entering the clinic without HB_sAg or anti-HB_s have a great risk of developing HB_sAg in a relatively short time, presumably due to environmental exposure and an increased susceptibility to infection.

(b) The patients do not, in general, make anti-HB_s before having HB_sAg and have a high probability of becoming persistent carriers of HB_sAg.

(c) There is evidence of mild to moderate chronic liver damage in the patients who have persistent HB_sAg.

(d) Patients entering with anti-HB_s, or who develop anti-HB_s, are protected against development of HB_sAg.

The disadvantages and possible advantages of becoming infected with hepatitis B virus have not yet been fully evaluated. At present, however, it appears that the results argue in favor of artificially stimulating the immune system to prevent infection with hepatitis B virus.

VACCINE STUDY DESIGN

There are at least three possible questions one can raise concerning the nature of the anti-HB_s protection:

(*i*) Is there a titer effect in the sense that higher antibody titer affords greater protection?

(*ii*) Is there a delay in the antibody response from the time of vaccination?

(*iii*) Is the antibody persistent or are boosters required?

Further, there is also the question of whether the anti-HB_s stimulated by the vaccine offers the same protection as anti-HB_s arising naturally. Initially, the discussion is contingent upon assumed answers to these questions. Specifically, the most optimistic assumptions will be made; namely, the vaccine will produce sufficient titer with minimal delay, remain persistent, and offer the same protection as naturally acquired anti-HB_s. Later these assumptions can be relaxed.

In a standard clinical trial, a randomly selected group of patients who, at the time of admission, have no anti-HB_s or HB_sAg receive the vaccine while a control group receives a placebo (or gamma-globulin). The experimental design problem is one of estimation of sample size in order to draw a conclusion concerning vaccine efficacy in a stated amount of time.

Hence, the traditional analysis involves testing the significance of the differences in infection rates given in Table 1 (column 7) and Table 2 (column 6). This analysis would tell whether the vaccine is at least as effective as natural anti-

Table 3. Sample sizes necessary for each group to detect a difference between patients with and without vaccination with constant error probabilities of 0.01

Months of exposure	Sample size
2	355
3	167
4	106
5	60
6	59
7	51
8	42
9	33
10	32
11	27
12	24

HB_s. The magnitude of the infection rate difference increases as the months of exposure increase. Therefore, the sample size necessary will be a decreasing function of time. Assuming constant error probabilities of 0.01, the sample sizes required are displayed in Table 3 and can be found using the methods of Natrella (13). The values tabulated refer to the number of patients in each group so that the total number of patients is twice the value given. For instance, if the trial were to last 9 months, a total of 66 patients would be required.

It is unlikely that there would ever be as many as 66 patients available at one time to initiate the trial at the clinic that we have monitored. In practice, a staggered start would be required, and this may necessitate a matched pair analysis. Thus, the elapsed time will exceed the 9 months and the matched pairs may introduce difficulties associated with the criterion for matching as well as the increased sample size that usually accompanies matched pair analyses (14). Clearly a superior plan would have everyone vaccinated. Of course, vaccinating everyone appears then to lose the notion of controls.

The model with the parameter $n = 1$, however, reduces to an exponential curve. Therefore, the conditional probability of not converting to HB_sAg in any month, given that the patient did not have HB_sAg in the previous month, equals $e^{-\lambda}$. Hence, the risk of converting in any given month is a constant independent of the time the patient has been on dialysis.

It is the time independence feature of the model that permits a study design that may not require a conventional control; for it is possible to use the data on the past and present patients as historical controls with the assurance that valid comparisons of future and past results are obtainable because of the constant risk over time. This analysis is based on the assumption that during the period of the trial the conditions for transmission of HB_sAg have not markedly changed from the time period on which the data analysis was based. For instance, a change in personnel or procedures could affect the time independence. However, personnel and procedural changes were made during the 4 years considered in this study, and the time independence was not affected. Hence, since the vaccination experiment would cover only a short period of time, the historical controls would appear appropriate.

Thus, a second trial wherein everyone is vaccinated is feasible if a conventional control cannot be used. In addition to satisfying a possible ethical problem, an advantage in sample size reduction is also obtained.

Using the parameter λ, it is apparent that the rate at which patients without anti-HB_s develop HB_sAg is approximately 8% ($= 1 - e^{-\lambda}$) per month. It is somewhat difficult to estimate the conversion rate for patients with anti-HB_s because so few patients converted. A conservative estimate would indicate that the rate is a maximum of 1% per month. If the rate were as high as 1% per month, then one would predict that at 12 months the probability of not developing HB_sAg would be approximately 0.887 when, in fact, the actual probability is 0.951.

Therefore, the test of hypothesis consists of determining whether it is more likely that the vaccinated population is infected at the 8% per month rate of those without anti-HB_s or at the 1% per month rate of those with anti-HB_s. Narula and Li (15) demonstrate that the sample size required to distinguish between the possibilities depends only on the ratio of the infection rates. This key ratio then would be 8 to 1. Again, assuming 0.01 error probabilities, a sample size (s) of nine patients would be sufficient, according to Eq. 2 of Narula and Li. The test statistic would reject the 1% per month hypothesis if

$$\hat{\lambda} > \frac{2s\lambda^*}{\chi^2(2s)}$$

where $\hat{\lambda}$ is the estimated λ, $\lambda^* = 0.01$, the hypothesized λ, and $\chi^2(2s)$ is the first percentile point of the chi-square distribution with $2s$ degrees of freedom. When $s = 9$, the boundary is 0.026.

A priori it would be reasonable to expect that the infection rate in the vaccinated group would lie between the two extreme rates of 8% and 1%. This middle ground could be reached under circumstances where one or more of the assumed answers to the questions concerning anti-HB_s protection was overly optimistic. For instance, there may be a titer effect in that greater antibody provides protection, and the vaccine may not produce adequate antibody in all individuals. With the hypothesis that the vaccinated patients have an infection rate of 4%, the critical ratio is 2 to 1, obtained by using the 8% and 4% rates. Using 0.01 error probabilities and the same calculations, a sample of 49 patients is required with the boundary for $\hat{\lambda}$ equal to 0.066 when $\lambda^* = 0.04$.

In practice, one does not usually specify an alternative hypothesis to the historical control of infection at the 8% rate. Hence, the sample size calculations do not lead to a direct plan. A reasonable procedure would be to follow nine patients until their infection rate exceeds the 0.026 boundary. If, after 9–12 months, the rate is less than 0.026, then one would conclude in favor of the hypothesis that the vaccinated patients are protected to the same degree as those who entered with or developed anti-HB_s. If the rate exceeds 0.026, then start a new group of 49 patients to test the intermediate hypothesis. If the rate for this group exceeds the 0.066 boundary, then one would conclude that the vaccine is not effective or that the population selected is unable to produce antibody at satisfactory levels. In any case, the number of patients and time to reach a conclusion should be less than the conventional clinical trial.

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