Intradermal Vaccination of Adults with Three Low Doses (2 μg) of Recombinant Hepatitis B Vaccine. II. Persistence of Immunity and Induction of Immunologic Memory

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Of the 110 dentists who had presented seroconversion 50 days after the intradermal application of three 2 μg doses of the Belgian recombinant vaccine against hepatitis B (HB), administered eight years before at an interval of one month between the 1st and 2nd doses and of five months between the 2nd and 3rd doses, 51 were included for the assessment of the persistence of immunity. None of the dentists had hepatitis or had received HB vaccine during this period. All subjects were submitted to serological tests for the detection of the following markers of hepatitis B virus (HBV) infection: HBsAg, anti-HBc, HBeAg, anti-HBe, and anti-HBs, with no HBsAg, anti-HBc, HBeAg or anti-HBe being detected. A microparticle enzyme immunoassay (MEIA) revealed the presence of anti-HBs at protective titers (≥ 10 mIU/ml) in 42 dentists (82.4%), with the anti-HBs titer being higher than 100 mIU/ml in 36 of them (70.6%) (good responders), between 10 and 100 mIU/ml in 6 (11.8%) (poor responders), and lower than 10 mIU/ml in 9 (17.6%) (non-responders). According to clinical data and serological tests, none of the dentists had presented disease or latent HBV infection during the eight years following the first vaccination. A 2 μg booster dose was administered intradermally to eight dentists with anti-HBs titers lower than 10 mIU/ml (non-responders) and to six dentists with titers ranging from 10 to 100 mIU/ml (poor responders); the determination of anti-HBs one month later demonstrated the occurrence of seroconversion in the eight non-responders and an increase in anti-HBs titer in the six poor responders. In summary, the present results demonstrated the prolonged persistence of protection against HBV infection and the development of immunologic memory provided by vaccination against HB – with intradermal application of three 2 μg doses of the Belgian recombinant vaccine at 0, 1, and 6 months – carried out eight years before in 51 dentists.

Key words: hepatitis B vaccine - seroconversion - immunologic memory

The immunogenicity and safety of hepatitis B (HB) vaccines – both recombinant and derived from human plasma administered intramuscularly in three doses at standard intervals (0, 1, and 6 months) – are similar and have been widely confirmed (Szmuness et al. 1980, 1982, Stevens et al. 1985, 1987, Zajac et al. 1986, Lee et al. 1995). In addition to high seroprotection rates, several studies have demonstrated prolonged persistence of immunity against HB (Hadler et al. 1986, Stevens et al. 1985, 1992, Ding et al. 1993, Greenberg 1993, Lieming et al. 1993, Tabor et al. 1993, Wainwright et al. 1997, Wu et al. 1999, 2001, Dexter et al. 2002, Whittle et al. 2002) and the development of immunologic memory (Gonzalez et al. 1993, Lai et al. 1993, Resti et al. 1993, Trivello et al. 1995, West & Calandra 1996, Da Villa et al. 1997, Li et al. 1998, Shih et al. 1999, Chada & Arankalle 2000, Chongsrisawat et al. 2000, Poovorawan et al. 2000, Ayerbe & Perez-Rivilla 2001, Banatvala et al. 2001, Watson et al. 2001, Dexter et al. 2002) as a result of the intramuscular application of both recombinant and human plasma-derived HB vaccines to adults and children using the scheme cited above. Banatvala et al. (2001) referred to four studies carried out with recombinant vaccines administered intramuscularly to adults and children using habitual doses and regimens, in which five to eight years after vaccination the serum anti-HBs titer was equal to or higher than 10 mIU/ml in 83% to 93% of the vaccinees. Watson et al. (2001), evaluating the response to intramuscular application of a booster dose of recombinant HB vaccine 13 years after the application of three usual doses at 0, 1, and 6 months, demonstrated the presence of immunologic memory in all individuals studied (18 vaccinated during childhood and seven vaccinated at more than 30 years of age).

However, only few and short-term studies are available regarding the duration of immunity conferred by HB vaccine administered by the intradermal route. Thus, the objective of the present study was to determine the persistence of immunity conferred by recombinant HB vaccine administered intradermally in three small doses of 2 μg (1/10 of the amount of antigen present in the usual dose indicated for adults). During the course of the study the presence of immunologic memory was also assessed.

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The vaccines were administered at the same intervals routinely employed in the application of intramuscular doses (0, 1, and 6 months). We studied dentists who had been immunized by intradermal injection of the Belgian recombinant vaccine eight years before.

PATIENTS AND METHODS

The initial objective of the present study was to determine the persistence of immunity against HB (presence of anti-HBs in serum at protective titers) in 110 dentists who had seroconverted eight years before after intradermal administration of three 2 µg doses of the Belgian HB recombinant recombinant vaccine (Engerix-B, SmithKline Beecham), applied at an interval of one month between the first and second doses and of five months between the second ant third doses. During the course of the study it became also possible to determine the presence of immunologic memory conferred by the first vaccination.

Telephone contact could be maintained with 106 of the 110 dentists, who were informed about the project to be developed, and all were willing to participate in the second part of the study. Fifty-five dentists were excluded: 26 had received one or more HB vaccine doses during the period mentioned; one died during the study; and 28 had received a fourth intradermal 2 µg dose of HB vaccine in 1991, six months after the third dose.

The study protocol was approved by the Bioethics Committee of the Regional University Hospital of Northern Paraná (HURNP), State University of Londrina.

The 51 dentists included in the study signed an informed consent form to participate after they had individually received explanations about the methodology and objectives of the study by one of the authors (MCME). All participants answered a brief questionnaire regarding identification data and information about the occurrence of viral hepatitis and vaccination against HB during the period since the first vaccination.

For the serological tests, blood samples (7 ml) were collected between October 5 and December 22, 1999 from 51 dentists by venipuncture in the antecubital region using a standard technique. The following serological markers of HBV infection were determined at the Laboratory of Clinical Analyses of HURNP by a microparticle enzyme immunoassay (MEIA) using kits from Abbott Laboratories, US: HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe, with the results only being characterized as reactive or non-reactive, except for anti-HBs which was quantified as mIU/ml, with the result being considered to be reactive when the anti-HBs concentration was equal to or higher than 10 mIU/ml (Hadler et al. 1986, CDC 1987). Dentists with reactive anti-HBs were considered good or poor responders when the serum anti-HBs concentration was higher than 100 mIU/ml or between 10 and 100 mIU/ml, respectively (Pead 1986, Ferraz et al. 1992). Intradermal application of a 2 µg booster dose (0.1 ml) of the Belgian recombinant vaccine between January 14, 1999 and January 21, 2000 was indicated to dentists who did not show any of the serological markers for hepatitis B virus (HBV) infection, or whose anti-HBs concentration was lower than 100 mIU/ml. About one month later between January 11 and February 18, 2000, a new blood sample was collected from dentists receiving this booster dose for the determination of all serological markers by MEIA, with quantification of anti-HBs.

The age of the participants is reported as mean and median. The Fisher exact test was used for the evaluation of serum anti-HBs concentration when associated with age group and sex, with the level of significance set at 0.05. The comparison between the age of the males and females was made using the Mann-Whitney test.

RESULTS

The age of the 51 dentists ranged from 31 to 65 years, with a mean age of 41.1 and a median of 39.0 years. The mean age of the 28 male dentists (43,4 years) and of the 23 female dentists (38,3 years) was similar ($p > 0.05$). Forty (78.5%) dentists were white and 11 (21.5%) were non-white (all of Asian origin).

None of the 51 dentists reported the occurrence of viral hepatitis during the period since the first vaccination, nor did they present HBsAg, anti-HBc, HBeAg or anti-HBe in serum. Nine (17.6%) dentists (6 male; 3 female) were non-reactive and 42 (82.4%) dentists (22 male; 20 female) were reactive for anti-HBs, with the anti-HBs concentration being 10 to 100 mIU/ml in 6 of them (11.8%) and higher than 100 mIU/ml in 36 (70.6%). Anti-HBs concentration was 10 to 100 mIU/ml in 6 (14.3%) of the 42 dentists reactive for this antibody and higher than 100 mIU/ml in 36 (85.7%); in the last group. 13/36 (36.1%) have serum anti-HBs titer higher than 1000 mIU/ml; among the 22 male dentists reactive for anti-HBs, the serum concentration of this antibody ranged from 10 to 100 mIU/ml in 3 (13.6%) and was higher than 100 mIU/ml in 19 (86.4%). Serum anti-HBs concentration ranged from 10 to 100 mIU/ml in 6 (15%) of the 20 female dentists reactive for this antibody and was higher than 100 mIU/ml in 17 (85%). No significant difference in the distribution of serum anti-HBs titers was observed between male and female dentists. Comparison of the number of individuals reactive for anti-HBs between the group with a serum anti-HBs concentration of 10 to 100 mIU/ml and the group with a concentration higher than 100 mIU/ml did not reveal any significant difference between sexes.

Twenty five (59.5%) of the 42 dentists reactive for anti-HBs ranged in age from 30 to 40 years, while 17 (40.5%) were older than 40 years, with neither of these two age groups showing a predominance in the number of dentists reactive for anti-HBs ($p = 0.4740$).

Eight dentists, who were non-reactive (< 10mIU/ml) for serum anti-HBs eight years after the first vaccination, were submitted to the intradermal application of a 2 µg booster dose of the Belgian recombinant vaccine; seroconversion was observed in all of them, with the serum anti-HBs titer ranging from 10 to 100 mIU/ml in 2 (25%) (poor responders) and with the titer being higher than 100 mIU/ml in 6 (75%) (good responders) (Table); a 2 µg booster dose of the same vaccine was also administered intradermally to six dentists with a serum anti-HBs concentration between 10 and 100 mIU/ml (poor responders) eight years after the first vaccination, with an increase in anti-HBs concentration being observed in all of them: 4

TABLE

Distribution of serum anti-hepatitis B (HBs) titers before and one month after intradermal administration of a 2 µg booster dose of the Belgian recombinant HB vaccine in the dentists in whom the serological test for anti-HBs carried out eight years after the first vaccination was non-reactive (< 10mIU/ml) and in whom the serum anti-HBs titers ranged from 10 to 100 mIU/ml

Serum anti-HBs titer before application of the booster dose (mIU/ml)	Serum anti-HBs titer one month after application of the booster dose (mIU/ml)							
	10 |—| 100		100 —|1000		> 1000		Total	
	Nr	%	Nr	%	Nr	%	Nr	%
< 10	2	25	4	50	2	25	8	100
10 |—| 100	—	—	4	66.7	2	33.3	6	100

(66.7%) with a titer between 100 and 1000 mIU/ml and 2 (33.3%) with a titer higher than 1000 mIU/ml (Table).

DISCUSSION

Determination of serological markers for HBV infection in the 51 dentists who had shown seroconversion eight years before after intradermal administration of three 2 µg doses of the Belgian recombinant vaccine at 0, 1, and 6 months demonstrated that none of them had been infected with HBV during this period and that 42 (82.4%) still presented a protective serum anti-HBs concentration, with 36 of them (70.6%) showing a titer higher than 100 mIU/ml.

As mentioned, prolonged persistence of immunity conferred by HB vaccines has been demonstrated for both recombinant vaccines and vaccines derived from human plasma administered intramuscularly at routinely recommended doses to adults and children at an interval of one month between the first and second dose and of five months between the second and third dose (0, 1, and 6 months). However, few studies have been published concerning the persistence of immunity conferred by HB vaccination with low doses administered intradermally using the same regimen. Bryan et al. (1992) demonstrated that protection (anti-HBs ≥ 10 mIU/ml) conferred by the application of three 2 µg doses of the North-American vaccine obtained from human plasma (Heptavax-B, Merck Sharp & Dohme) in the same regimen as used here (0, 1, and 6 months) persisted in 97% of vaccinees for 14 months and in 89% for 25 months; application of a booster dose in the 16 individuals who had not seroconverted induced protective serum anti-HBs levels in 69%. Jaiswal et al. (1995) showed the persistence of immunity in 93.3% of 200 adults who had seroconverted three years before, after intradermal application of three 4 µg doses of human plasma vaccine in the same regimen as cited above; a booster effect resulting from the intradermal administration of a complementary dose (4 µg) of the same vaccine was observed in 27 individuals who had become non-reactive. Kurugöl et al. (2001) reported the persistence of immunity in 87% of 44 children who had developed immunity five years before after intradermal administration of three 2 µg doses of the French recombinant vaccine (GenHevac B, Pasteur-Mérieux) at the same intervals (0, 1, and 6 months); seven children who had become non-reactive (anti-HBs < 10 mIU/ml) showed a serum concentration of this antibody higher than 1000 mIU/ml after intramuscular administration of an additional vaccine dose.

In the present study, 14 dentists who had seroconverted after the first vaccination, but who had become non-reactive eight years later or who presented a serum anti-HBs concentration between 10 and 100 mIU/ml, responded to intradermal application of a booster dose of the same vaccine (2 µg), all of them returning to present protective anti-HBs titers, which were higher than 100 mIU/ml in 12 individuals (Table). This fact represents clear evidence of the presence of immunologic memory induced by the first vaccination eight years before. The authors cited above (Bryan et al. 1992, Jaiswal et al. 1995, Kurugöl et al. 2001), who reported a shorter persistence of immunity to hepatitis B than that observed in the present study, i.e., two, three, and five years after the first vaccination, respectively, also observed a booster effect after intradermal application of a booster dose to previously seropositive individuals whose serum anti-HBs titer had been lower than 10 mIU/ml. As mentioned, evidence of the induction of immunologic memory by a first vaccination has also been obtained after intramuscular vaccination with usual doses of recombinant and human plasma-derived vaccines.

The serum anti-HBs titers quantified by MEIA eight years after the first vaccination were found to be very high (higher than 1000 mIU/ml) in 11 (26.2%) of the 42 dentists who had been considered to be good responders and in 2 (22.2%) of the nine dentists considered to be poor responders after the first vaccination, eight years before, suggesting that these individuals may be recently exposed to an antigen stimulus (professional exposure to hepatitis B virus?).

Additionally, high serum anti-HBs concentrations (640 and > 1000 mIU/ml) were observed in two (50%) dentists – one poor responder and one non-responder upon serological testing eight years after the first vaccination – of the four considered to be poor responders after the first vaccination (eight years before), injected intradermally with a 2 µg booster dose of the same vaccine.

The data obtained in this study permit us to conclude that the Belgian hepatitis B vaccine administered intradermally in three 2 µg doses (0, 1, and 6 months), in addition to causing high seroconversion rates, induces per-

sistent immunity lasting at least eight years and the establishment of immunologic memory.

The prolonged persistence of immunity and the development of immunologic memory in individuals receiving the complete HB vaccination scheme demonstrated in the literature and in the present study support the widely accepted concept that the administration of a booster dose of the HB vaccine becomes unnecessary in immunocompetent individuals who develop immunity after application of the basic regimen (three doses at appropriate intervals) (European Consensus Group on Hepatitis B Immunity 2000, Watson et al. 2001, Koff 2002).

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