NOTES

Successful Memory Response following a Booster Dose with a
Virosome-Formulated Hepatitis A Vaccine Delayed Up to 11 Years

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Received 27 August 2010/Returned for modification 30 December 2010/Accepted 2 March 2011

Boosting adult travelers with the virosome-formulated, aluminum-free hepatitis A vaccine Epaxal up to 128 months after a single primary dose confers full protection against hepatitis A, even in travelers aged 50 years and above. Delaying the booster dose did not influence the immune memory response to Epaxal.

Adults traveling from regions where the prevalence of hepatitis A is low to regions where it is high are at risk of acquiring symptomatic hepatitis A virus (HAV) infection (1). Long-lasting protection against HAV is, by recommendation, achieved with administration of 2 vaccine doses 6 to 18 months apart (12). In practice, many travelers do not return to see their doctors within the recommended time for the second (booster) dose, and therefore, defining the maximum interval between the two doses still conveying long-term seroprotection is of importance. Several studies using an aluminum-absorbed HAV vaccine (Havrix) and comparing various lengths of delay between the two doses still conveying long-term seroprotection is of importance. Several studies using an aluminum-absorbed HAV vaccine (Havrix) and comparing various lengths of delay of the second dose (≥24 months [9] or up to 8 years [8] after a single primary dose) have shown comparable memory responses irrespective of the interval between the two doses. A single dose of Epaxal, the only aluminum-free virosomal HAV vaccine currently available, is highly immunogenic (3). Two doses of Epaxal administered 12 months apart give adults a real-time protection of at least 9 to 11 years, which is predicted to last for at least 30 years in ≥95% of individuals (4).

A study in 1999 showed that Epaxal is highly immunogenic when a booster is given 18 to 54 months after the primary dose, indicating that a delay in the administration of the booster of up to 54 months does not lead to loss of immunogenicity (2). A subsequent study in 2006 investigated the immunogenicity of an Epaxal booster administered ≥5 years after the primary immunization. This report presents the results from that 2006 study but as a combined analysis of both the 1999 and 2006 studies and evaluates the level of memory response to Epaxal when given as a booster dose 9 to 128 months (0.8 to 10.7 years) after the primary dose. Previously unpublished results from the 1999 study evaluating the postbooster immune response in a subgroup 9 to 17 months after the primary immunization are also included.

Both studies were noncomparative, open-label, and single-center studies and were performed at the Swiss Tropical and Public Health Institute (STPH) in Basel, Switzerland; they were approved by the Ethics Committee of Basel EKBB (Basel, Switzerland) and conducted in compliance with the Declaration of Helsinki. All subjects provided informed consent before study entry.

The study population included subjects who had received Epaxal primary immunization at the STPH travel clinic but had not received a booster dose for ≥9 months (in the case of the 1999 study) or ≥5 years (in the case of the 2006 study). The exclusion criteria were as previously described (2). All participants received a booster dose of 0.5 ml Epaxal (containing ≥24 IU of HAV antigen; Crucell Switzerland AG) supplied in ready-to-use syringes and given intramuscularly into the deltoid muscle.

HAV antibody concentrations (mIU/ml) were measured in parallel in paired serum samples that were obtained for both studies at baseline and 4 to 7 weeks after the booster dose, using an enzyme immunoassay, AxSYM HAVAB 2.0 (Abbott). Seroprotection cutoffs of ≥20 mIU/ml and ≥10 mIU/ml are presented, both of which are accepted as HAV protection cutoffs (6). Additionally, the 6-mIU/ml cutoff is presented as the lowest measurable concentration of specific anti-HAV antibodies by this sensitive assay, validated at the Department of Virology, Max von Pettenkofer Institute, Ludwig Maximilians University, Munich, Germany.

Descriptive statistics were used for data analysis. Seroprotection rates and geometric mean concentrations (GMCs) of HAV antibodies were evaluated by booster interval (9 to 29 months, 30 to 41 months, 42 to 54 months, and 98 to 128 months), by the age of the subjects (<50 years and ≥50 years), and by their gender. The time interval effect on the HAV antibody response and the pre- versus postbooster HAV anti-
body concentration correlations were calculated using logistic regression analysis.

Overall, 130 subjects were analyzed, i.e., 104 from the 1999 study (booster interval, 9 to 54 months), whose samples were still available for retesting, and 26 from the 2006 study (booster interval, 98 to 128 months). The mean age was 39.3 years (range, 20.5 to 73.0 years) for the whole group, 33.4 years (range, 20.5 to 48.0 years) for the subgroup of 50-year-old subjects (n = 100), and 59.1 years (range, 50.0 to 73.0 years) for the subgroup of ≥50-year-old subjects (n = 30). There were more females (n = 72) than males (n = 58).

The proportions of seroprotected subjects across the booster time intervals and according to age group and gender are presented in Table 1. The majority (73.8%) of subjects tested 9 to 128 months after receiving the primary dose of Epaxal still had measurable anti-HAV antibody concentrations (≥6 mIU/ml is the lower limit of detection), and 59.2% still had protective levels of anti-HAV antibodies (≥10 mIU/ml). The

| Table 1. Number of seroprotected subjects by booster interval, age, and gender |
|---|
| Visit | Conc of Epaxal (mIU/ml) | Booster interval (mo) | Age | Gender |
| | | 9–29 | 30–41 | 42–54 | 98–128 | <50 yr | ≥50 yr | Females | Males |
| Prebooster | 6 | 33 (73.3) | 25 (78.1) | 17 (63.0) | 21 (80.8) | 96 (73.8) | 78 (78.0) | 18 (60.0) | 59 (81.9) | 37 (63.8) |
| | ≥10 | 29 (64.4) | 21 (65.6) | 13 (48.1) | 14 (53.8) | 77 (59.2) | 63 (63.0) | 14 (46.7) | 53 (73.6) | 24 (41.4) |
| | ≥20 | 20 (44.4) | 19 (59.4) | 8 (29.6) | 13 (50.0) | 60 (46.2) | 49 (49.0) | 11 (36.7) | 44 (61.1) | 16 (27.6) |
| Postbooster | All three cutoffs | 45 (100.0) | 32 (100.0) | 27 (100.0) | 130 (100.0) | 100 (100.0) | 72 (100.0) | 58 (100.0) |

* Booster interval, 9 to 128 months.

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| Table 2. Geometric mean concentrations of anti-HAV antibodies by booster intervala |
|---|
| Booster interval (mo) | Subjects | Visit | No. of subjects | GMC (mIU/ml) | 95% CIs | Fold increase |
| | | | | | | |
| 9–29 | All | Prebooster | 45 | 16 | 10, 24 | NA |
| | | Postbooster | 45 | 1,397 | 1,009, 1,934 | 90 |
| | Age <50 yr | Prebooster | 35 | 19 | 12, 29 | NA |
| | Postbooster | 35 | 1,528 | 1,090, 2,142 | 82 |
| | Age ≥50 yr | Prebooster | 10 | 9 | 3, 23 | NA |
| | Postbooster | 10 | 1,022 | 404, 2,588 | 120 |
| 30–41 | All | Prebooster | 32 | 23 | 14, 37 | NA |
| | | Postbooster | 32 | 1,685 | 1,147, 2,481 | 75 |
| | Age <50 yr | Prebooster | 26 | 26 | 16, 44 | NA |
| | Postbooster | 26 | 1,825 | 1,233, 2,701 | 70 |
| | Age ≥50 yr | Prebooster | 6 | 12 | 3, 44 | NA |
| | Postbooster | 6 | 1,199 | 362, 3,978 | 99 |
| 42–54 | All | Prebooster | 27 | 11 | 6, 18 | NA |
| | | Postbooster | 27 | 1,262 | 829, 1,920 | 120 |
| | Age <50 yr | Prebooster | 23 | 10 | 6, 17 | NA |
| | Postbooster | 23 | 1,185 | 781, 1,798 | 123 |
| | Age ≥50 yr | Prebooster | 4 | 18 | 4, 86 | NA |
| | Postbooster | 4 | 1,812 | 417, 7,867 | 103 |
| 98–128 | All | Prebooster | 26 | 24 | 14, 41 | NA |
| | | Postbooster | 26 | 2,115 | 1,379, 3,245 | 89 |
| | Age <50 yr | Prebooster | 16 | 34 | 17, 65 | NA |
| | Postbooster | 16 | 2,117 | 1,284, 3,489 | 63 |
| | Age ≥50 yr | Prebooster | 10 | 14 | 5, 37 | NA |
| | Postbooster | 10 | 2,113 | 835, 5,349 | 155 |
| 9–128 (all subjects) | All | Prebooster | 130 | 17 | 13, 22 | NA |
| | | Postbooster | 130 | 1,557 | 1,285, 1,886 | 91 |
| | Age <50 yr | Prebooster | 100 | 19 | 15, 25 | NA |
| | Postbooster | 100 | 1,590 | 1,301, 1,943 | 83 |
| | Age ≥50 yr | Prebooster | 30 | 12 | 7, 20 | NA |
| | Postbooster | 30 | 1,451 | 863, 2,439 | 123 |

* Geometric mean concentrations (GMCS), including 95% confidence intervals (CIs), were calculated from logarithmically transformed titer values. NA, not applicable.
prebooster seroprotection rates were, for each cutoff level, relatively similar across the different time intervals, indicating a remarkable long-term persistence of antibody levels up to 128 months after the priming immunization. A 100% postbooster seroprotection level was achieved in all interval groups.

Prebooster seroprotection rates were lower in the older (≥50) than the younger (<50) age group (47% versus 63% at ≥10 mIU/ml), but both age groups achieved 100% postbooster seroprotection in all time intervals. Females, as expected and previously reported (7, 9), had up to three times higher antibody concentrations than males (data not shown). Females also had higher prebooster seroprotection rates than males (74% versus 41% at ≥10 mIU/ml), but both groups achieved 100% postbooster seroprotection in all time intervals (Table 1). The GMC increased from 17 to 1,557 mIU/ml for the total population (Table 2). There were no significant differences in postbooster anti-HAV GMCs between older and younger subjects, and the time intervals did not influence the memory response in either of the two age groups (Table 2). Logistic regression analysis revealed that there were no statistically significant differences in antibody concentrations between the 4 booster interval subgroups (P = 0.1381) (Fig. 1; Table 2) and that low prebooster antibody concentrations correlated significantly with lower postbooster values (r = 0.59; P < 0.0001) (data not shown).

The present evaluation of 130 travelers aged 21 to 73 years demonstrates that a delay of the booster dose of up to 128 months after receipt of the primary vaccination does not influence the memory response to Epaxal. All subjects, even those ≥50 years old with lower prebooster anti-HAV antibody concentrations than the younger subjects, achieved 100% postbooster seroprotection irrespective of the time interval between the primary and booster vaccination. The proportional postbooster increases in GMCs were comparable between older and younger subjects, and high and nearly identical GMCs were obtained in all groups, even in the group of ≥50-year-old subjects, which had the highest proportion of subjects with no-longer-detectable specific anti-HAV antibodies (<6 mIU/ml) prior to the booster.

To our knowledge, the 2006 study reports on the largest group of subjects published to date who had received a booster dose after a considerably long interval (8.2 to 10.7 years). The observation that the time interval between primary and booster dose does not influence the immunogenicity of the booster dose confirms the published findings of the 1999 study (2) and is in line with the findings of other studies using an aluminum-adsorbed hepatitis A vaccine (8, 9). This antibody memory recall response indicates that the first vaccine dose elicits an efficient priming of the immune system via an early proliferative T-cell response, known from natural HAV infection (13) and observed following immunization with an aluminum-adsorbed hepatitis A vaccine (5).

The results of the present study confirm the observation from other studies that although lower prebooster seroprotection rates are found in older subjects (>40 years old) than in younger subjects, the postbooster immune responses are comparable between the different age groups (7, 10, 11).

The present findings are of special importance for clinical practice, as travelers frequently do not return for the scheduled hepatitis A booster vaccination after 6 to 18 months. The fact that a delayed booster dose does not impede the memory response to Epaxal offers more flexibility for hepatitis A vaccination schedules for travelers.

This work was supported by Crucell Switzerland AG (Berne, Switzerland). C. Hatz has received honoraria for presenting scientific data from Crucell-Berna Biotech, GSK, and Novartis; R.V.D.P., B.R.B., G.F., and M.H. have no conflict of interest to declare. C.H. is an employee of Crucell Switzerland AG.

Sonja Basta, a medical and scientific writer (Canada), provided assistance in preparing and editing the manuscript.

REFERENCES
1. Askling, H. H., L. Rombo, Y. Andersson, S. Martin, and K. Ekdahl. 2009. Hepatitis A risk in travelers. J. Travel Med. 16:233–238.
2. Beck, B. R., C. Hatz, R. Brommimann, and C. Herzog. 2003. Successful booster antibody response up to 54 months after single primary vaccination with virosome-formulated, aluminum-free hepatitis A vaccine. Clin. Infect. Dis. 37:e126–e128.
3. Bovier, P. A. 2008. Epaxal: a virosomal vaccine to prevent hepatitis A infection. Expert Rev. Vaccines 7:1141–1150.
4. Bovier, P. A., et al. 2010. Predicted 30-year protection after vaccination with an inactivated virosome hepatitis A vaccine. J. Med. Virol. 82:1629–1634.
5. Cederna, J. B., D. Klinzman, and J. T. Stapleton. 1999. Hepatitis A virus-specific humoral and cellular immune responses following immunization with a formalin-inactivated hepatitis A vaccine. Vaccine 18:892–898.
6. Centers for Disease Control and Prevention. 2006. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. 55:1–23.
7. D’Acremont, V., C. Herzog, and B. Genton. 2006. Immunogenicity and safety of a virosomal hepatitis A vaccine (Epaxal) in the elderly. J. Travel Med. 13:78–83.
8. Darrow, S., M. Lindh, and L. Widerstrom. 2004. Excellent booster response 4 to 8 years after a single primary dose of an inactivated hepatitis A vaccine. J. Travel Med. 11:120–121.
9. Landry, P., S. Tremblay, R. Darioli, and B. Genton. 2000. Inactivated hepatitis A vaccine booster given ≥24 months after the primary dose. Vaccine 19:399–402.
10. Nalini, D. R., et al. 1993. Worldwide experience with the CR326F-derived inactivated hepatitis A virus vaccine in pediatric and adult populations: an overview. J. Hepatol. 18(Suppl. 2):S51–S55.
11. Reuman, P. D., P. Kubilis, W. Hurni, L. Brown, and D. Nalin. 1997. The effect of age and weight on the response to formalin inactivated, alum-adsorbed hepatitis A vaccine in healthy adults. Vaccine 15:1157–1161.
12. World Health Organization. 2000. Hepatitis A vaccines—WHO position paper. Wkly. Epidemiol. Rec. 75:38–44.
13. Zachoval, R., M. Kroener, M. Brommer, and F. Deinhardt. 1990. Serology and interferon production during the early phase of acute hepatitis A. J. Infect. Dis. 161:353–354.

FIG. 1. Scatterplot of log-transformed hepatitis A virus (HAV) antibody concentrations after booster vaccination, as a function of time between the primary and booster doses.