

## Evaluation of inactivated hepatitis A vaccine in Canadians 40 years of age or more

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**Objective:** To assess the side effects and immune responses after three serial doses of a new inactivated hepatitis A vaccine in people 40 years of age or more.

**Design:** Open, noncomparative trial.

**Setting:** A hospital, a regional laboratory and public health units in British Columbia.

**Participants:** A volunteer sample of 64 healthy adults aged 40 to 61 years who were seronegative for hepatitis A virus (HAV). All were staff or associates of the health facilities. Exclusion criteria included elevated serum alanine and aspartate aminotransferase levels, a history of liver disease and recent travel to areas of high risk for HAV infection.

**Intervention:** A formalin-inactivated, alum-adsorbed vaccine containing 720 ELISA (enzyme-linked immunosorbent assay) units of antigen from HAV strain HM175 per 1.0-mL dose was injected intramuscularly into the deltoid area. The second and third doses were given 1 and 6 months later respectively.

**Main outcome measures:** A detailed diary of any adverse effects for 3 days after each dose. HAV antibody levels in blood samples taken before and 30 days after each dose.

**Results:** All subjects completed the planned series of vaccinations and blood tests; symptom diaries were returned after 190 (99%) of 192 vaccinations. Local symptoms, most often soreness, were reported after 46% of the vaccinations but were mild and usually resolved within 24 hours. A temperature of more than 38.0°C was never reported. Seroconversion occurred in all cases after the two primary doses, and the subjects were still seropositive at 6 months. After the booster dose the geometric mean titre was 2380 mIU/mL, all values being 200 mIU/mL or greater.

**Conclusion:** In healthy adults 40 years of age or more the HAV vaccine was well tolerated and highly immunogenic. Final antibody levels were much higher than reported in people passively immunized against HAV with immune serum globulin.

**Objectif :** Évaluer les effets secondaires et les réactions immunologiques après l'administration de trois doses en série d'un nouveau vaccin inactivé de l'hépatite A chez les personnes de 40 ans ou plus.

**Conception :** Essai non comparatif ouvert.

**Cadre :** Un hôpital, un laboratoire régional et des services sanitaires de Colombie-Britannique.

**Participants :** Échantillon composé de 64 adultes volontaires de 40 à 61 ans séronégatifs au virus de l'hépatite A (VHA). Toutes ces personnes faisaient partie du personnel des établissements de santé ou leur étaient associées. Parmi les critères d'exclusion, mentionnons les taux sériques élevés d'alanine-aminotransférase et d'aspartate-amino-

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transférases, des antécédents de maladie du foie ou un voyage récent vers des régions à risque élevé d'infection au VHA.

**Intervention :** Injection intramusculaire d'un vaccin adsorbé sur alun et inactivé au formol, contenant 720 unités ELISA (titrage avec immuno-adsorbant lié à une enzyme) d'antigène du VHA de la souche HM175 par doses de 1,0 mL, dans la région du deltoïde. Les doses deux et trois ont été données 1 et 6 mois plus tard respectivement.

**Principales mesures de résultat :** Journal détaillé de tout effet secondaire indésirable pendant 3 jours après l'injection de chaque dose. On a mesuré les concentrations d'anticorps du VHA dans les prélèvements sanguins avant et 30 jours après chaque dose.

**Résultats :** Tous les sujets ont subi la série prévue de vaccins et de prélèvements sanguins; les journaux symptomatiques ont été remis après 190 (99 %) des 192 vaccinations. On a signalé des symptômes locaux, la plupart du temps des douleurs, après 46 % des vaccinations, mais il s'agissait de douleurs bénignes qui se dissipaient habituellement dans les 24 heures. Personne n'a signalé une température dépassant 38°C. Il y a eu séroconversion dans tous les cas après les deux premières doses, et les sujets demeuraient séropositifs après 6 mois. À la suite de la vaccination de rappel, le titre moyen géométrique était de 2 380 mUI/mL, toutes les valeurs étant d'au moins 200 mUI/mL.

**Conclusion :** Chez les adultes sains de 40 ans ou plus, le vaccin VHA a été bien toléré et s'est avéré fortement immunogène. Les taux ultimes d'anticorps étaient beaucoup plus élevés que ceux constatés chez les personnes immunisées passivement contre le VHA par immunoglobuline sérique.

**H**epatitis A poses a substantial health risk for people living in or travelling to developing countries.<sup>1</sup> In other areas hepatitis A occurs when local sanitation measures are ineffective as a result of a natural disaster, inadequate sewage disposal systems or fecal contamination of surface waters and shellfish. Day-care facilities are potential foci for spread of hepatitis A among children, parents and staff.<sup>2</sup> The disease can affect groups of addicts using parenteral drugs<sup>3</sup> and homosexual men.<sup>4</sup> Contact with a person who has hepatitis accounts for about 26% of cases in the United States;<sup>5</sup> however, in most reported cases the source of exposure is unknown.<sup>1</sup>

Prevention of hepatitis A is possible with the administration of pooled immune serum globulin. To be fully effective it must be given within 2 weeks after exposure or before one enters a high-risk environment. The dose (0.02 to 0.06 mL/kg) can make immunization an unpleasant experience. Protection wanes after a few months.

Recent advances in the in-vitro propagation of the hepatitis A virus (HAV)<sup>6</sup> have led to the development of several candidate vaccines, based on inactivated virus, attenuated live virus or recombinant subunit proteins.<sup>1</sup> Most progress has been made with inactivated vaccines,<sup>7,8</sup> the methods being similar to those used to produce inactivated poliovirus vaccine. A vaccine containing HAV strain HM175, propagated in MRC5 fibroblasts and inactivated with formalin, is being assessed in clinical trials.<sup>9</sup> In this study we focused on the responses of older adults to hepatitis A vaccine, because this age group responds less well than younger groups to certain other viral vaccines such as hepatitis B vaccine.<sup>10</sup>

## Methods

Study participants were recruited from the staff of British Columbia's Children's Hospital, Vancouver, the Vancouver facility of the Canadian Red Cross Blood Transfusion Service and local public health units. Participants had to be healthy and 40 years of age or more. They were excluded if they had detectable antibodies to HAV, elevated serum alanine and aspartate aminotransferase (ALT/AST) levels on routine testing, a history of liver disease or excessive alcohol consumption, or liver enlargement or right upper quadrant tenderness on examination, or if they had travelled within 3 months to an HAV-endemic area.

Written informed consent was obtained from all subjects before enrolment. The study design was approved by the Ethics Committee of the University of British Columbia and conformed with provisions of the Declaration of Helsinki and its amendments.

The inactivated hepatitis A vaccine used in this study was manufactured with tissue-culture-derived virus produced by SmithKline Beecham Biologicals, Rixensart, Belgium.<sup>9</sup> It was purified by ultrafiltration and gel filtration and inactivated with formaldehyde. The lot used (VHA 005A4) conformed to the standard specification and was formulated to contain not less than 720 ELISA (enzyme-linked immunosorbent assay) units of hepatitis A antigen per 1.0-mL dose, adsorbed onto 0.5 mg of aluminum hydroxide. Phenoxyethanol was added as a preservative.

The vaccine was injected intramuscularly in the deltoid region with a 23-gauge needle 2.5 cm in length. Two nurses gave most of the vaccines, at intervals of 0, 1 and 6 months. Participants were

asked to complete a symptom diary in the evening after each vaccination and for the 3 subsequent days, an interval previously established as appropriate both to detect adverse effects and to observe their resolution.<sup>9</sup> Comments were solicited regarding any headache, malaise, loss of appetite, nausea, vomiting, local soreness, redness, induration or swelling; comments regarding other symptoms were also permitted. Subjects were asked to grade any symptoms as mild (present but easily tolerated), moderate (causing enough discomfort to interfere with normal activities) or severe (preventing normal activities). Oral temperature was to be measured daily with a digital thermometer supplied for the purpose.

A blood sample of 10 mL was taken on the day of screening (about 14 days before the first vaccination) and 1, 2, 6 and 7 months after initial vaccination. The screening sample was tested for serum ALT and AST levels with the use of an automated assay by the hospital biochemistry laboratory; an ALT level of 45 U/L or more was considered elevated.<sup>11</sup> The screening samples were also tested for anti-HAV antibodies with a commercially available enzyme immunoassay (Havab, IMx System, Abbott Laboratories Limited, Mississauga, Ont.) by the British Columbia Center for Disease Control, Vancouver. All samples were tested for total HAV antibody titres with an ELISA inhibition assay developed at SmithKline Beecham Biologicals. Subjects with a titre of less than 20 mIU/mL were considered to have a seronegative status. An increase in the antibody titre to 20 mIU/mL or more was considered to be evidence of seroconversion.

Descriptive statistical analyses included calculation of the geometric mean antibody titre at each point when samples were taken, distribution of individual antibody titres at each point and rates of reported adverse reactions.

## Results

Of the 118 volunteers who were screened, 80

were HAV seronegative and had normal serum ALT and AST levels. Sixty-four agreed to participate in the study. All were between 40 and 61 years of age (mean 47.7 [standard deviation 6.07] years); 23 were men, mostly physicians, and 41 were women, mostly nurses and technicians. All of the subjects completed the planned series of vaccinations and blood tests.

No allergic reactions were observed. The subjects completed and returned their symptom diaries after 190 (99%) of the 192 vaccinations, the only omissions occurring after dose 3. No severe adverse effects were reported, nor did any subject require medical care for any symptoms.

The incidence rates of local reactions are summarized in Table 1. The overall rate was 46%. Soreness was most frequent, reported after 82 (43%) of the doses; it was mild in 77 (94%) of the cases and moderate in 5 (6%). Most (66%) of the affected subjects noted that the soreness had lasted less than 24 hours after vaccination. An area of redness 10 mm or more in diameter was reported after seven doses (4%). Other local symptoms occurred infrequently. The severity of reactions did not increase with successive doses.

The incidence rates of solicited general symptoms are in Table 2. The subjects were diligent about recording their daily temperatures: only 51 (7%) of the 760 readings were omitted. At least one reading during the first 24 hours after vaccination was documented in 184 instances (97%). A temperature of 38.0°C was reported after 3 (2%) of the 184 vaccinations for which measurements were submitted. No higher values were recorded.

Headache was the most frequently reported symptom (in 28 [15%] of the cases) but was usually mild. The background rate of headaches in this population was not determined. Malaise was reported after 23 vaccinations (12%) and was graded as mild in 22. Nausea was reported in 13 cases (7%). Eleven of the episodes of nausea were rated as mild; no one reported vomiting. Decreased appetite was reported in 3 cases (2%).

Table 1: Incidence rates of local adverse reactions to a new hepatitis A vaccine in people 40 years of age or more

Symptom	Dose; no. (and %) of subjects		
	1 (n = 64)	2 (n = 64)	3 (n = 62)
Soreness	33 (52)	25 (39)	24 (39)
Duration < 24 h	26 (41)	16 (25)	12 (19)
Duration ≥ 24 h	7 (11)	9 (14)	12 (19)
Redness	5 (8)	4 (6)	6 (10)
Area diameter < 10 mm	3 (5)	2 (3)	3 (5)
Area diameter ≥ 10 mm	2 (3)	2 (3)	3 (5)
Induration	2 (3)	1 (2)	1 (2)
Swelling	1 (2)	1 (2)	2 (3)
Any symptom	34 (53)	26 (41)	27 (44)

The desired timetable for the vaccinations and the collection of blood samples was met for 57 (89%) of the 64 subjects after doses 1 and 2 and for 55 (86%) after dose 3. The principal analysis of anti-HAV responses involved these subjects. The seroconversion rates and geometric mean anti-HAV titres of these subjects are given in Table 3. Seroconversion occurred in all cases after the two primary doses of vaccine, and the subjects remained seropositive at 6 months (before dose 3). After the booster dose the geometric mean titre for the group increased more than ninefold. Over 60% of the anti-HAV titres after dose 3 exceeded 2000 mIU/mL.

The responses of the subjects excluded from the analysis of the anti-HAV responses were not appreciably different from those of the other subjects. Seroconversion occurred in all of them after dose 2, the titres varying from 42 to 2295 mIU/mL. The subject with the low titre became seronegative before dose 3 but had a subsequent titre of 251 mIU/mL. The titres after dose 3 ranged from 251 to 9570 mIU/mL. Using data from all the subjects, the geometric mean titre after dose 3 was 2369 mIU/mL,

only slightly lower than that for the main group (2380 mIU/mL).

## Discussion

The participants in this trial were very cooperative in adhering to the schedule of vaccinations and blood tests and in returning fully completed symptom diaries for each period of observation. The fact that most were physicians and nurses might have resulted in a more exacting and minute assessment of symptoms than is typical of people outside the health care profession. Despite this potential bias, volunteers reported only minor adverse effects after vaccination.

The principal local adverse effect, soreness, might have resulted from the aluminum hydroxide used to adsorb the virus particles. However, the soreness was rated as mild in all but five instances, and most cases resolved within 24 hours. No severe local reactions were reported.

Systemic adverse effects were reported after 22% of the vaccinations. Subjects faithfully recorded daily temperatures, yet no instance of one greater

Table 2: Incidence rates of general symptoms after hepatitis A vaccination

Symptom	Dose; no. (and %) of subjects		
	1 (n = 64)	2 (n = 64)	3 (n = 62)
Headache	15 (23)	6 (9)	7 (11)
Duration < 24 h	10 (16)	4 (6)	2 (3)
Duration ≥ 24 h	5 (8)	2 (3)	5 (8)
Malaise	10 (16)	7 (11)	6 (10)
Duration < 24 h	5 (8)	3 (5)	3 (5)
Duration ≥ 24 h	5 (8)	4 (6)	3 (5)
Nausea	8 (12)	4 (6)	1 (2)
Fever (temperature ≥ 38.0°C)	2 (3)	1 (2)	0
Appetite loss	1 (2)	2 (3)	0
Any symptom	22 (34)	9 (14)	10 (16)

Table 3: Immune responses of eligible subjects\* after hepatitis A vaccination

Response	Before dose 1 (n = 57)	After dose 1 (n = 57)	After dose 2 (n = 57)	Before dose 3 (n = 55)	After dose 3 (n = 55)
No. (and %) of seropositive subjects	0	51 (89)	57 (100)	55 (100)	55 (100)
Geometric mean antibody titre, mIU/mL	0	164	288	253	2 380
Antibody titre, mIU/mL, no. (and %) of subjects					
< 20	57 (100)	6 (11)	0	0	0
20- < 200	0	32 (56)	16 (28)	21 (38)	0
200- < 2000	0	18 (32)	39 (68)	34 (62)	21 (38)
≥ 2000	0	1 (2)	2 (4)	0	34 (62)
Maximum titre, mIU/mL	0	2 080	3 000	1 542	58 385

\*Received all three doses of vaccine and provided all blood samples as required.

than 38.0°C was reported. Our supplying the volunteers with digital thermometers might have contributed to the high compliance rate. Headache was the most frequently reported symptom, followed by malaise. Unfortunately, baseline rates of these symptoms in the study group were not available to indicate whether a higher incidence was reported after vaccination. Other systemic symptoms were reported infrequently. All general symptoms were rated by the participants as nonsevere. None resulted in work loss or necessitated medical assessment. It may be relevant that the number of symptom reports, particularly those of headache and nausea, decreased more than 50% after dose 1. This may have reflected greater apprehension among the subjects receiving the first dose or concurrent winter-time illness, because the first dose was given between December and February.

Immune responses to HAV were strong in this group: seroconversion occurred in all cases after dose 2. Antibody levels declined minimally before the third dose, following which they increased almost 10-fold. All subjects included in the analysis had an antibody titre of 200 mIU/mL or greater after dose 3. Such levels greatly exceed those observed after routine doses (0.02 mL/kg) of immune serum globulin, which have been shown to provide significant protection against hepatitis A.<sup>12</sup> Unfortunately, the minimum level of neutralizing antibody required to prevent HAV infection is unknown. It appears that only low levels of serum neutralizing antibody to HAV are needed to prevent infection.<sup>13</sup> In a group of 23 adults passively immunized with immune serum globulin the geometric mean titre 5 days after vaccination was 21 mIU/mL.<sup>14</sup> All of the vaccinees in our study had an anti-HAV titre of 20 mIU/mL or greater after the second dose. Among 21 people found to be HAV seropositive due to natural infection,<sup>14</sup> the geometric mean titre was 3485 mIU/mL. One-quarter of the vaccinees in our study had a titre of equal or greater value after dose 3. The highest titre was 58 385 mIU/mL.

## Summary

In healthy adults 40 years of age or more the HAV vaccine was well tolerated and highly immunogenic. Its good performance in all age groups has led to its recent licensure in several European countries. Licensure is expected in Canada and the United States within 2 years. The vaccine will be of greatest interest to frequent international travellers, sparing them from repeated courses of immune serum globulin. Studies of accelerated schedules for convenient vaccination of travellers are in progress. Other groups for whom this vaccine might be indicated include the armed forces, international relief or

volunteer organizations and people moving to developing countries. In Canada this vaccine might find application within the health care profession, in isolated communities with poor sanitation and possibly among students and staff of day-care facilities. Combined preparations with hepatitis B vaccine are being assessed, a measure that would facilitate use among people at risk.

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