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Comparison of the Reactogenicity and Immunogenicity of Two Different Dose Levels of Hepatitis A Vaccine in Healthy Children and Adolescents

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SUMMARY **An open study was performed to compare the reactogenicity and immunogenicity of an inactivated hepatitis A vaccine administered in two different doses and schedules to 460 healthy volunteers aged 3-18 years. Participants were randomized to two groups to receive either two doses of 720 ELISA Units (EL.U) inactivated hepatitis A per 0.5 ml dose according to a 0,6-month schedule, or three doses of 360 EL.U according to a 0, 1, 6-month schedule. Transient local injection soreness was the most commonly reported symptom in almost half of both groups with no serious adverse events. One month after the primary course (one dose of 720 EL.U and two doses of 360 EL.U), 99% of 720 EL.U vaccinees had seroconverted, compared with 100% seroconversion in the 360 EL.U group. All vaccinees were seropositive after the booster dose of both vaccines with geometric mean anti-HAV titers of 2,359 and 2,967 mIU/ml in the 720 EL.U and 360 EL.U groups, respectively. The vaccine containing 720 EL.U of antigen per dose offers the advantage of convenience and acceptance of immunization afforded by a two-dose course of vaccination accompanied by a comparable antibody response with that achieved after three doses of vaccine containing 360 EL.U of antigen per dose.**

Poor hygiene, overcrowding and inadequate sanitation are the major environmental conditions for the most important route of transmission of HAV, the fecal/oral route. In such conditions, the infection occurs early in life and is almost always subclinical.[1] Where standards of hygiene and sanitation are improved, there is a shift in epidemiology, with an increase in the average age of infection and a decrease in the prevalence of antibodies against the virus.[2] Changes in seroepidemiologic patterns of hepatitis A (HAV) in recent years are attributed to general improvements in living standards. For the individual, active immunization can provide long-term protection against HAV infection; from a public health perspective, active immunization controls this disease effectively.[3] Active immunization of children against hepatitis A became a reality in 1993, when the first pediatric hepatitis A vaccine was licensed. The initial schedule consisted of a two dose primary vaccination course with 360 ELISA Units (EL.U) of inactivated hepatitis A per dose followed by a booster dose six months later. To achieve earlier protection, reduce the number of doses and thus improve convenience, a vaccine formulation with twice the antigen load, i.e. 720 EL.U, per dose is now recommended for single dose primary immunization followed by a booster dose between 6 and 12 months later. If such a regimen is to be implemented, the major criterion of its success will be an antibody response of comparable magnitude to that achieved with the 3 x 360 EL.U schedule without any demonstrable increase in reactogenicity. Previous studies have shown both dose levels of the vaccine to be immunogenic in

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children and adolescents.[4-9] Thus, we undertook a study to compare the reactogenicity profile of inactivated hepatitis A vaccine containing 720 EL.U per dose administered according to a 0, 6-month schedule to that following a three dose course of vaccination (0, 1, 6-months) with a vaccine containing 360 EL.U inactivated hepatitis A antigen per dose in healthy children and adolescents. A secondary objective was to compare the seroconversion rates and geometric mean titers (GMTs) of antibodies to HAV (anti-HAV) elicited by the two dose levels of antigen after the primary course and the booster dose.

MATERIALS AND METHODS

Four hundred and sixty children and adolescents (aged 3 to 18) were enrolled into this open study at two sites (Khon Kaen; center 1, Bangkok; center 2) in Thailand after written, informed consent was obtained from their parents/guardians. The study was conducted in accordance with the provisions of the Declaration of Helsinki as amended in Hong Kong in 1989 and Good Clinical Practice Guidelines in operation at the time of initiation of the study.

Exclusion criteria included prevaccination serum positive for anti-HAV; elevated serum liver enzymes (ALT); any history of significant and persisting hematologic, hepatic, renal, cardiac, or respiratory disease; chronic alcohol consumption; any chronic drug treatment including immunosuppressive therapy; history of allergic disease likely to be stimulated by any vaccine component and simultaneous administration of any other vaccine(s) or any immunoglobulin during the study period.

All subjects had their medical history taken and underwent physical examination. Eligible children/adolescents were then randomly assigned to one of two groups, randomization being carried out at each site separately by random allocation of preassigned patient accession numbers.

The inactivated hepatitis A vaccine (Havrix™) was manufactured by SmithKline Beecham Biologicals. Group 1 received 720 ELISA (enzyme-linked immunosorbent assay) units (EL.U) of inactivated hepatitis A per 0.5 ml dose administered according to a single primary dose with a booster dose six months later (0, 6-month schedule). Group 2 received 360 EL.U per 0.5 ml dose administered according to a two-dose primary course, with a booster dose six months after the initial vaccination (0, 1, 6-month schedule). The purified viral suspension was inactivated with formaldehyde and adsorbed onto 0.5 mg aluminium hydroxide. The residual amount of formaldehyde in each dose was not more than 0.1 mg/ml. Different batches of vaccine were employed at the two sites. Vaccines were administered into the deltoid muscle.

On the day of vaccination and for three subsequent days, local symptoms (soreness, redness, swelling) and general symptoms (fever defined as body temperature > 37.5°C, headache, malaise, loss of appetite, nausea and vomiting) as well as any other findings were recorded by the vaccinee or his/her parent or guardian on diary cards. In one study center the general symptom 'malaise' was not solicited.

Blood samples were obtained 7 to 14 days before the first vaccination and were tested in the investigators' laboratory for the presence of anti-HAV using the commercially available ELISA assay HAVAB by Abbott Laboratories (Chicago, USA) and liver enzyme activities (ALT and AST) by standard spectrophotometric assays. Serum specimens obtained at months 1, 2, 6 and 7 were tested for anti-HAV using a commercial ELISA (Boehringer Enzymun Kit)[10] calibrated with a World Health Organization international standard reference serum and expressed in milli-international units per milliliter (mIU/ml). The assay cut-off is set at 33 mIU/ml, which corresponds with the lower quantitation limit of the test; therefore, subjects with titers below 33 mIU/ml were considered seronegative.

The primary objective of this study was to compare the reactogenicity of the two vaccine regimens. Compilation of data from the two centers provided a sample size sufficient to reach 80% statistical power with a type I error fixed at 5% which would allow detection of a two-fold increase in local adverse event rates occurring at an incidence of 10%. An enrollment of this size would also allow the detection of an approximate 5% difference in seroconversion and a 20% difference in GMTs. To verify the statistical validity of pooling data from the two centers, the categorical linear model was used to compare the ratio of males to females, the overall incidence of symptoms and seroconversion rates. The general linear model was used to compare anti-

body titers between vaccine regimens and centers taking into account main effects (group, center and sex) and their interaction. Pooled data from the two centers for each dose level were compared using an alternative to Bernard's test performed on Stat Xact-3 to calculate an exact two-sided p-value for testing the null hypothesis that "the difference in the incidence of symptoms following vaccination with either antigen dose is equal to '0'" and "the difference in seroconversion rates following vaccination with either antigen dose is equal to '0'." The analysis of reactogenicity was bipolar in that two analyses were performed: the first, based upon the number of doses followed by symptoms and, the second, based upon the number of subjects reporting symptoms in the two groups. Analysis of variance was used to compare anti-HAV GMTs between groups, centers and gender.

RESULTS

No significant interaction between group, center and sex was found for mean ages. Hence it could be determined that with respect to key demographic data, the centers could be pooled (Table 1). Comparison of mean ages between groups and centers and sex ratio between groups showed no statistically significant difference; therefore, both groups could be considered for comparative analysis.

No statistically significant differences were found in the reactogenicity profiles of the two vaccine regimens based upon the number of doses which gave rise to symptoms (Table 2). Soreness at the injection site, reported after 46.0% of 720 EL.U doses and 47.3% of 360 EL.U doses, was the most frequently reported local symptom, and headache, reported after 15.7% of 720 EL.U doses and 17.4% of 360 EL.U doses, the most prevalent general symptom. Although slightly more 360 EL.U doses were followed by malaise (17.7%), this result is misleading in that this symptom was not solicited in one of the centers.

When reactogenicity analysis was performed based upon the number of subjects in the two groups reporting symptoms over the course of the study, significantly more subjects in the 360 EL.U/dose group reported symptoms overall, injection site soreness as well as headache, malaise and loss of appetite (Table 2).

Table 1 Subject distribution and demography of the total study population

	Group 1 (vaccinated with 720 EL.U/dose) (N=230)		Group 2 (vaccinated with 360 EL.U/dose) (N=230)	
No. of subjects	Center 1 N=80	Center 2 N=150	Center 1 N=81	Center 2 N=149
Mean age and range (years)	11.3 ± 3.55		11.2 ± 3.67	
	Center 1 11.1 ± 3.85	Center 2 11.4 ± 3.40	Center 1 10.8 ± 4.05	Center 2 11.5 ± 3.45
Gender ratio (M/F)	87/143		68/162	
	Center 1 41/39	Center 2 46/104	Center 1 28/53	Center 2 40/109

Statistics: Analysis of variance compared mean ages in pooled demographic data and showed:
No significant difference in mean ages between groups (p=0.4741)
No significant difference in mean ages between centers (p=0.34)
A significant difference in mean ages between sexes (p=0.0008)
No interaction between: group,sex,center (p=0.4741)
group,sex (p=0.5745)
sex,center (p=0.2137)
group,center (p=0.4952)

Table 2 Percentage of doses leading to reported symptom/ Percentage of vaccinees reporting symptoms

	Group 1 (720 EL.U/dose)	Group 2 (360 EL.U/dose)	p value
No of documented doses/subjects→	452/230	673/228	
With symptoms (solicited/unsolicited): doses/subjects			
	58.4/77.4	56.6/85.5	0.5583/0.0385
Solicited local injection site symptoms: doses/subjects			
Soreness	46.0/62.6	47.3/73.7	0.6912/0.0164
Redness	12.6/23.5	13.1/22.8	0.8408/0.3848
Swelling	7.3/14.3	7.4/17.5	0.9543/0.4388
Solicited general symptoms: doses/subjects			
Fever	5.5/10.4	4.8/12.7	0.6506/0.5396
Headache	15.7/27.0	17.4/36.8	0.5111/0.0030
Loss of appetite	3.5/7.0	6.1/13.6	0.1095/0.0434
Malaise*	13.7/23.3	17.7/32.4	0.1962/0.0230
Nausea	2.0/3.9	3.4/8.3	0.2988/0.1102
Vomiting	0.2/0.4	1.0/3.1	0.3482/0.1386

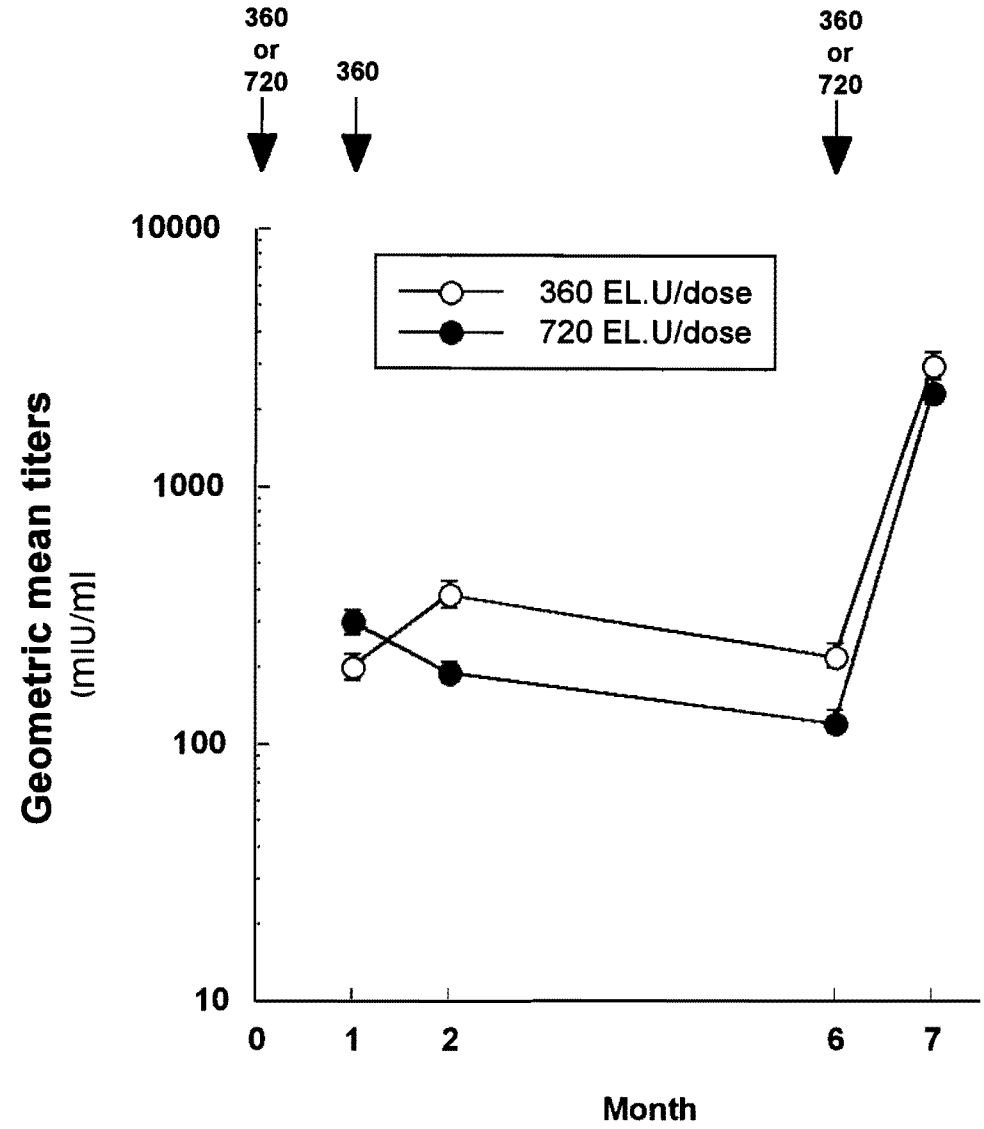
*Malaise was not solicited in one center.

Table 3 Serocoversion rates over the course of the study

Time (months)	Group 1 (720 EL.U/dose)		Group 2 (360 EL.U/dose)	
	No.	%	No.	%
1	227/230	98.7	216/228	94.7
2	220/229	96.1	226/226	100.0
6	202/222	91.0	214/217	98.6
7	221/221	100.0	214/214	100.0

Most solicited local injection site and general symptoms were described as easily tolerated in both vaccine groups and all solicited adverse events in both groups resolved within the 4-day follow-up period after vaccination. No serious adverse events were reported in either group during the study period.

Table 3 details the seroconversion rates over the course of the study. No statistically significant difference was detected in seroconversion rates one month after the primary course of both regimens, *i.e.*, month 1 in the 720 EL.U group (one dose primary course) versus month 2 in the 360 EL.U group (two dose primary course): $p = 0.4889$. However, a second dose of the vaccine containing one half the antigen dose, administered one month after the first dose of the two-dose primary course of vaccination, elicited statistically significantly higher rates of seroconversion at months 2 (100%,



Note: Arrows indicate vaccine dose.

Fig. 1 Geometric mean anti-HAV titers elicited by the two vaccine regimens over the course of the study

p = 0.0264) and 6 (98.6%, p = 0.0030) than that after the single primary dose of 720 EL.U at the same time points (96.1% and 91.0%, respectively). Figure 1 provides graphic representation of geometric mean anti-HAV titers elicited by the two regimens. Statistically significant lower GMTs of anti-HAV antibodies were observed in subjects who received the vaccine containing 720 EL.U antigen per dose after the primary course (p = 0.0025) and after the booster dose of both regimens (p = 0.0277). Statistically significant differences were also shown between sexes and centers at all time points when GMTs were compared laterally using analysis of variance model (p = 0.0001 in all cases); however, these differences were not consistent in that one center or sex did not always exhibit higher GMTs than the other. One month after the booster dose, all vaccinees were seropositive for anti-HAV anti-

bodies with an approximate 8-fold increase in GMTs in both groups compared with that achieved one month after completion of the primary vaccine course (month 1 in the 720 EL.U group and month 2 in the 360 EL.U group).

DISCUSSION

This study shows that the increased antigen per dose does not influence the reactogenicity either locally or generally. Based on the reactogenicity analysis of the number of doses followed by symptoms, the vaccine with the higher antigen load gave rise to local and general side effects of the same type, intensity and duration as those induced by the vaccine of exactly the same composition but one-half the antigen dosage. The local injection site symptoms most likely resulted from the alum adjuvant,[11] the concentration of which is identical in the 720- and 360 EL.U formulations. The incidence of systemic adverse events was similar to that reported in other studies following vaccination with the inactivated hepatitis A vaccine, with headache being the most frequent.[12-15] According to the secondary reactogenicity analysis which compared the number of vaccinees with symptoms over the course of the study, the vaccine schedule with 720 EL.U/dose was less reactogenic than the vaccine schedule employing 360 EL.U/dose. This observation is logical in that subjects who received the vaccine containing 720 EL.U/dose received one injection less.

Both vaccines employed in this study induced a satisfactory immune response in this cohort as indicated by 100% seropositivity for antibody to the vaccine antigen with GMTs greater than or equal to that previously reported following a full course of vaccination with hepatitis A vaccine in healthy children and adolescents.[4-9, 12-15] The statistical power of the study enrollment allowed detection of a 5% difference in seroconversion rates, a difference which would be clinically significant as well, *ie*, a difference of this proportion would impact on vaccine response. No statistically significant difference was determined in seroconversion one month after one dose of vaccine irrespective of antigen dose, which indicates that within the limits of this study, the greater antigen load did not elicit earlier antibody response. However, Findor *et al.*[14] reported a rapid appearance of high titer antibodies to HAV within 15 days of the first dose of 720 EL.U in children aged 2 to 13 years. Lee *et al.*[7] reported similar results in 9-18 year olds. The significantly higher seropositivity rates at months 2 and 6 in the group vaccinated with two doses of 360 EL.U as seropositivity waned following the single primary dose of 720 EL.U are probably not relevant given the similarity in seroconversion after the primary course of both regimens. Moreover, all vaccinees in both groups were seropositive one month after the booster dose of both vaccines. Finally, there is also evidence that previous exposure to hepatitis A virus will protect against disease even when the antibody titers have become undetectable.[16] Thus protection against hepatitis A may also be apparent in vaccinated subjects whose anti-HAV titers at month 6 are no longer measurable.

Although statistically significant, the differences in GMTs between the vaccines is probably not clinically significant given that the statistical power of the sample size allowed a significant difference to be detected with a difference of 20% in GMTs. In other words, a difference of this proportion would not affect the protective efficacy of the vaccine or the kinetics of decrease of antibody titers following vaccination with either dose level. Innis *et al.*[17] reported a cumulative protective efficacy of 95% with the vaccine containing 360 EL.U/dose in a 17-month follow-up of 40,119 children aged 1 to 16 years. At month 8 of a 0, 1, 12 month course, the GMT was 200 mIU/ml. Van Damme *et al.*[18] evaluated the persistence of anti-HAV after vaccination with the vaccine containing 720 EL.U/dose administered according to a 0, 1, 6-month schedule in healthy adults. In a 5 year follow up, they reported a 60% decrease in titers within the first year, followed by a 14% decrease during the second year. From month 48 to month 60 the GMT decreased by about 27%. Similar antibody kinetics have been observed after vaccination with 1,440 EL.U of antigen per dose administered according to both 0, 6 and 0, 12-month schedules.[17] According to this model, antibodies in both groups could be expected to persist for at least 20 years. Wiedermann *et al.*[19] reported on persistence rates of an early hepatitis A vaccine containing either 180 or 360 EL.U of antigen per dose prepared from HAV strains CLF based on an observation period of 7 years. Although the vaccination schedule was 0, 1, 2, 12 months, results showed almost the same antibody kinetics up to 1 month after booster vaccination and after the booster the GMTs were practically identical.

In conclusion, the inactivated hepatitis A vaccine containing

720 ELISA Units of antigen per dose offers the advantage of better tolerability, convenience and acceptance of immunization afforded by a two-dose course of vaccination as evidenced by the significant differences in reactogenicity analysis on a per subject basis. Thus, higher coverage can be achieved. Moreover, the accompanying antibody response after two doses compares favorably with that achieved after three doses of the vaccine containing 360 ELISA Units of antigen per dose.

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