IMMUNOGENICITY AND SAFETY OF AN INACTIVATED HEPATITIS A VACCINE AMONG SINGAPOREANS

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Abstract. The immunogenicity and reactogenicity of an inactivated hepatitis A virus (HAV) vaccine was studied in healthy Singaporean adult volunteers.

One hundred and forty healthy volunteers with normal alanine (ALT) and aspartate (AST) transaminases and no previous exposure to HAV, received three 1 ml doses (720 ELISA units) of an inactivated HAV vaccine (Smithkline Beechams Biologicals) following a 0, 1, 6 months vaccination schedule. All subjects were asked to record and grade the severity of any reactions for three consecutive days after each dose. Serum ALT and AST as well as anti-HAV were measured at 0, 1, 2, 6 and 7 months after the first vaccine dose. Anti-HAV seroconversion occured when levels rose above 40 mIU/ml.

Eighty-five percent of vaccinees seroconverted after the first innoculation and 99% after the second injection. All vaccinees seroconverted after the third dose. Geometric mean anti-HAV titers (GMTs) were, respectively, 119, 391, 4406 mIU/ml one month after each of the three doses. The most common side effect was transient pain and tenderness at the vaccination site. No elevation of ALT or AST levels were noted during the study period.

The inactivated hepatitis A vaccine used in this study is safe and highly immunogenic in the local adult population. Two doses one month apart appeared to give adequate protection.

INTRODUCTION

Over the past decade, the incidence of hepatitis A virus (HAV) infection in Singapore has been declining, due primarily to improved hygiene and sanitary conditions. The sero-prevalence for HAV among Singaporeans under the age of 20 years is now zero and HAV appears to be no longer an infection in children and adolescents (Yap and Guan, 1993). The young adults, who form a significant proportion of the Singaporean population, are therefore at risk of getting primary HAV infection, particularly as they continue to travel to and from highly endermic areas in the region and to a lesser extent when they ingest shell fish which may be contaminated. In time, more and more Singaporean adults will have no natural immunity; clinical hepatitis will occur with exposure and the threat of HAV epidemics will be a real one. The need for hepatitis A vaccination is therefore both real and urgent in this age group.

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A multi-center study to establlish the immunogenicity and reactogenicity of an inactivated hepatitis A vaccine in the local adult population was performed recently.

MATERIALS AND METHODS

Vaccine

The candidate inactivated vaccine (lot no VHA046A4) was prepared from the HM175 strain of HAV (SmithKline Beecham Biologicals) as described previously (Andre *et al*, 1990). Three 1 ml doses (each dose contained 720 enzyme-linked immunosorbent assay units or EL U) were injected intramuscularly into the deltoid muscle according to a 0, 1, 6 months vaccination schedule.

Subjects

Volunteers participating in this study were from all walks of life, and were all less than 40 years old.

Inclusion criteria included good health, as determined by history taking and physical examination at trial entry. Volunteers were excluded from the study if they had a history of liver disease, chronic alcohol consumption, clinical signs of acute disease at entry, hepatomegaly, abnormal alanine and aspartate transaminases, and antibodies to hepatitis A virus (anti-HAV).

Written consent was obtained from all volunteers and the study was conducted in accordance with the provisions of the Declaration of Helsinki. The clinical protocol was approved by the Medical Clinical Research Committee of the Ministry of Health of Singapore.

Study design

This was an open study using 720 EL U of the above mentioned vaccine given intramuscularly at 0, 1 and 6 months on volunteers who were tested negative for HAV antibody.

On the day of vaccination (3 and 8 hours post vaccination) and once daily for three consecutive days, all subjects were required to record any local or systemic reactions and grade these reactions according to a four point none, mild, moderate, severe scale.

Blood samples for serum levels of liver enzymes and anti-HAV were obtained from each volunteer before and at 1, 2, 6 and 7 months after the first vaccine dose.

Biochemical and serological testing

Serum alanine (ALT) and aspartate (AST) transaminases were measured using standard laboratory techniques.

Total antibodies HAV (anti-HAV, IgM plus IgG) were measured by enzyme-immunoassay (HAVAB R EIA; Abbot Laboratories. North Chicago, Illinois, USA) (Duermeyer *et al*, 1978). To quantitate the anti-HAV level, the assay was performed using a standard reference obtained from the World Health Organization. To detect titers exceeding 150 mIU/ml, the procedure utilizes 10 µl of test serum/control volume for incubation with the HAV (human) - coated polystyrene bead in the presence of 200 µl of anti-HAV : horseradish peroxidase conjugate. To determine titers less than 150 mIU/ml, the assay was modified to use 100 µl of test serum/control and 100 µl of anti-HAV : horseradish peroxidase conjugate instead. Subjects with antibody titers less than 40 mIU/ml were considered seronegative. Seroconversion occurred when anti-HAV level rose above 40 mIU/ml.

Statistical analysis

Chi-squared analysis, the Mann-Witney U test and Student's *t*-test were used for comparison of results where appropriate.

RESULTS

One hundred and forty subjects received all three injections and came for all the required blood tests. There were 53 males and 87 females. Their ages ranged from 18 to 39 years (mean age 25 years). There were 113 Chinese, 10 Malays, 13 Indians and 4 Eurasians.

The proportion of responders and geometric mean titers (GMTs) of the vaccinees are summarized in Fig 1. Eighty-five percent (119/140) of the volunteers seroconverted after the first dose, and 99% (138/140) after the second dose. One hundred percent sero-conversion was achieved after the third dose at six months. GMT rose progressively with each dose of vaccine and were, respectively, 119, 391, 4406 mIU/ml one month after each of the three doses. There were no differences between the anti-HAV seroconversion rates of males compared to females at the three time points although female subjects gave higher GMTs at month seven ($p < 0.010$) (Tables 1, 2).

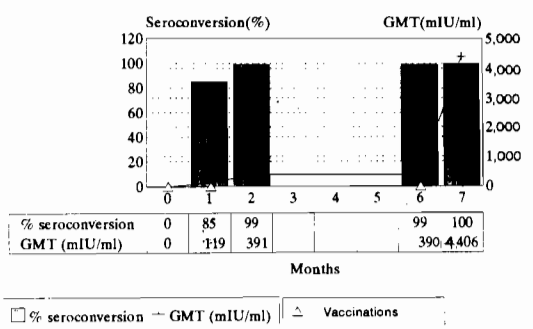

Fig 1–Immunogenicity of an inactivated HAV vaccine.

Table 1

Geometric mean titers (GMT) of anti-HAV following vaccination with an inactivated HAV vaccine.

Months after first dose		0	1	2	6	7
GMT mIU/ml	females (n = 87)	0	133	440	524	5,445*
	males (n = 53)	0	95	323	241	3,112*

* $p < 0.010$

Table 2

Percent seroconversion to anti-HAV following vaccination with an inactivated HAV vaccine.

Months after first dose		0	1	2	6	7
% seroconversion	females (n = 87)	0	91	97	99	100
	males (n = 53)	0	76	100	100	100

The most common side effect was transient pain and tenderness at the site of innoculation. This was reported in 24% of vaccinees after the first innoculation, in 21% after the second and in 21% after the third. One patient developed fits after the first innoculation but was later discovered to be an epileptic on regular medications. No serious systemic reactions were reported. Headache and malaise were the most frequently reported general symptoms. The frequency of occurrence after the first vaccine dose was 8% and 13% respectively. After the second dose, the percentage was 3% for both symptoms. After the third dose, the frequency was 5% for both symptoms. No elevation of alanine or aspartate aminotransferase levels were noted at various time points during the study period. In general, the vaccine was well tolerated and the vaccinees continued their regular activities without interruption.

DISCUSSION

This study demonstrated that the HM175 strain of inactivated hepatitis A vaccine manufactured by SmithKline Beecham Biologicals is safe and highly immunogenic in the local adult population and confirms earlier reports (Just and Berger, 1992; Goubau *et al*, 1992; Davidson *et al*, 1992) on the same vaccine.

Experience with passive immunization against hepatitis A has shown that protection could be achieved for at least 3 weeks after the administration of 0.015 ml/kg body weight of a hyperimmune globulin (100 mIU/ml) at a calculated titer of about 10 mIU/ml. This is much lower than levels found after vaccination (Wiedermann *et al*, 1990; Stapleton *et al*, 1985).

A single dose of the HAV vaccine used in this trial was shown to give a seroconversion rate of 85% and a GMT of 119 mIU/ml. Two doses of the vaccine were adequate in inducing a 99% seroconversion rate and a GMT of 390 mIU/ml. Protection against hepatitis A can therefore be achieved by giving two doses of this inactivated vaccine one month apart. It has indeed been shown that the vaccine is effective even if the interval between the first and second dose is reduced to only two weeks (Muller *et al*,1991). It has been suggested that should protection be needed within two weeks, passive-active vaccination could be considered (Tilzey *et al*, 1992).

The efficacy of this vaccine in the prevention of infection by HAV however was not assessed in the above trial. Studies in children were carried out in Thailand (Innis *et al*, 1994) and in the United States (Werzberger *et al*, 1992) to address this issue and results have been very encouraging. A 94% protective efficacy was achieved at the end of 12 months in the Thai study (n = 40, 119) whilst in the American study (n = 1037), the efficacy during a seasonal outbreak of hepatitis A was 100%.

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