IMMUNOGENICITY AND SAFETY OF A NEW INACTIVATED HEPATITIS A VACCINE IN THAI YOUNG ADULTS

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Abstract. In view of the increasing median age of hepatitis A virus (HAV) infection observed recently in Asia, and the resulting increased number of symptomatic cases occurring in adults, with the concomitant risk of outbreaks, immunization against this agent on a national scale might be considered. An open clinical trial was conducted in Thai adolescents and young adults in order to establish the immunogenicity and safety of a new inactivated hepatitis A vaccine. At 24-week intervals, two doses (primary dose and booster) of the hepatitis A vaccine (160 antigenic units per dose) were administered to 80 HAV-seronegative healthy volunteers, their ages ranging from 16 to 25 years. Local and systemic reactions were recorded within the first 7 days after each injection. Anti-hepatitis A virus antibody concentrations were measured by a modified radioimmunoassay before and one month after each injection. No serious adverse reactions were reported. Local reactions were confined to transient pain at the injection site, occurring within 24 hours after injection in 42.5% of the subjects after the first dose and 24.1% of the patients after the booster dose. Systemic reactions (particularly asthenia or myalgia) were observed in 35.0% and 8.9% of subjects after the first and the booster injection, respectively. Most of these reactions were transient. One month after the first dose, all 78 formerly seronegative subjects had attained satisfactory seroconversion levels of anti-HAV antibody concentrations (≥ 20mIU/ml) which they maintained until the booster. The booster dose elicited a 21-fold increase of HAV antibody levels, with a geometric mean titer of 2,964 mIU/ml (95% CI, 2,467-3,560), indicative of long-term protection. This new inactivated hepatitis A vaccine appears to be safe and highly immunogenic upon administration of a primary dose followed by a booster dose after 24 weeks. In countries where socio-economic improvement has postponed hepatitis A infection from early childhood (mostly asymptomatic) towards adolescence and adulthood, with the symptoms increasing in severity, inclusion of inactivated hepatitis A vaccine in a preventive vaccination program might be of benefit.

INTRODUCTION

Hepatitis A virus (HAV) may induce an acute, necro-inflammatory infection of the liver (Lemon, 1985; Forbes and Williams, 1990). The virus, classified within the genus *Hepatovirus* of the Picornavirus family, has a worldwide distribution. The most common mode of HAV transmission is by the fecal-oral route. While the incidence of infection decreases with age, the severity of clinical symptoms increases. In young children, hepatitis A infection is usually asymptomatic or mild, whereas in subjects above the age of 5 years, the infection is symptomatic in 75% of the cases (Hadler and McFarland, 1986). The complications and case-fatality ratio are age-dependent with a 4% mortality among reported cases in adults over 60 years (Forbes and Williams, 1988), but complications also occur in young adolescents and adults. A recent study on an outbreak of hepatitis A among young adults in the United States has shown that young healthy persons were also at risk for contracting severe complications (Willner *et al,* 1998).

Epidemiological patterns reflect the standards of hygiene and sanitation of the respective population in which the virus spreads (Gust, 1992), and thus areas of low, intermediate and high endemicity can be differentiated. The worldwide improvements in living standards, hygiene and sanitation during the last 20 years have led to a decreased incidence of HAV infection. This has caused an epidemiological shift of HAV susceptibility towards older age groups. An increasing number of children and young adults lack immunity to the residually circu-

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lating HAV, especially in areas of intermediate endemicity. In newly industrialized countries, such as Singapore and Taiwan, studies of HAV antibody levels in the population showed a low prevalence among young children (Wu *et al,* 1993; Yap and Guan, 1993). Likewise, antibody prevalence among children in Thailand has considerably declined during the last ten years (Innis *et al,* 1991; Poovorawan *et al,* 1993; 1997a, b). Consequently, the clinically manifested illness is found more frequently. Outbreaks of hepatitis A may occur as a consequence of fecal contamination of food or water as has been reported in Shanghai (Halliday *et al,* 1991). More limited outbreaks may occur among the institutional residents, for example prisoners or army recruits, as well as children attending day-care centers or boarding schools (Severo *et al,* 1997; Thacker *et al,* 1992).

In addition to standards of hygiene and sanitation, other etiologic factors have been found to influence on the incidence of hepatitis A, in particular attendance of day-care centers, drug addiction, homosexuality, or travelling to or working in areas of high endemicity. The American Advisory Committee on Immunization Practices (ACIP) recommends vaccination in these at-risk populations. Furthermore, in patients with chronic hepatitis, hepatitis A infection may further aggravate the pathologic alterations of hepatic function (Vento *et al,* 1998) and hepatitis A vaccination would be important in this context (ACIP, 1996).

AVAXIM™ is a new, inactivated hepatitis A vaccine developed by Pasteur Mérieux Connaught (PMC). The vaccine is derived from the GBM virus strain, and adapted by successive passages on the MRC-5 human diploid cell-line (Flehmig, 1981). The virus is then inactivated with formalin and aluminium hydroxide is added as adjuvant. During its clinical development, this hepatitis A vaccine, containing 160 HAV antigen units, has shown good immunogenicity and safety in adults (Vidor *et al,* 1996). PMC hepatitis A vaccine is currently licensed in more than 40 countries, including all European countries and Canada. Post-marketing surveillance has shown the safety of this vaccine to be satisfactory.

The aim of the present study was to evaluate the safety and immunogenicity of the PMC inactivated hepatitis A vaccine in HAV-seronegative healthy adolescents and young adults, when administered intramuscularly as one primary injection followed by a booster 24 weeks later.

MATERIALS AND METHODS

Study population and design

During 1996, 119 healthy young adults, aged from 16 to 30 years whose health condition and medical history were compatible with vaccination, were recruited in the Chulalongkorn Hospital to participate in this monocentric, open, non-controlled study. Each subject gave a blood sample at this screening visit to check hepatitis A serological status by enzyme-linked immunosorbent assay (ELISA) and for quantitative anti-HAV antibody determination. No more than 14 days later, only those subjects who were found to be HAV seronegative according to the ELISA screening test were included in the study (n = 80) and received the first dose of vaccine. Subjects returned to the clinic 28 days later for blood sampling and a safety evaluation. A booster dose of vaccine was given 24 weeks after the first dose. Blood samples were taken for antibody determination immediately prior to booster and 28 days later.

Vaccine

Each dose of vaccine (AVAXIM™; Pasteur Mérieux Connaught, Lyon, France) contained 160 antigen units of inactivated hepatitis A virus, 0.3 mg aluminium hydroxide, 2.5 µl phenoxyethanol, 12.5 µg formaldehyde, and up to 0.5 ml of medium 199 and water for injection.

The vaccine was presented in 0.5-ml pre-filled syringes (batch S3139). Injections were performed intramuscularly into the deltoid muscle.

Laboratory test

HAV-antibody pre-enrollment screening: Subjects' serum samples were screened for the presence of anti-HAV antibodies using a qualitative commercially available ELISA kit (Abbott Laboratories, North Chicago, Illinois, USA) at the Viral Hepatitis Research Unit, Faculty of Medicine, Chulalongkorn University, Thailand.

HAV-antibody titration (immunogenicity) : Anti-HAV antibody concentrations were measured before vaccination, at day 28, at week 24 (before

the booster dose) and at week 28 (28 days after the booster dose). Anti-HAV antibody levels were determined using a commercial radio-immunoassay (RIA) (HAVAB®, Abbott Laboratories, North Chicago, Illinois, USA) kit, modified in order to increase the sensitivity (Miller *et al,* 1993). Results were converted into international units using a reference curve generated from WHO Reference Standard (Gerety *et al,* 1983) and antibody concentrations were expressed in mIU/ml. The lower limit of detection of the modified RIA was 7 to 10 mIU/ml. All the serological titrations were done by BARC Laboratories, Ghent, Belgium, under the supervision of the PMC Clinical Sero-Immunology Laboratory (Val de Reuil, France). Seroconversion was defined as a rise in HAV antibody titers from < 20 mIU/ml before vaccination to ≥ 20 mIU/ml thereafter.

Safety analysis

Subjects were observed for any immediate reactions occurring within 15 minutes of each injection. Local reactions at the injection site and systemic reactions occurring between 15 minutes to 7 days after each injection were recorded by the subjects on self-monitoring forms that were then checked by the investigator at a follow-up visit 28 days after vaccination. The solicited local reactions at the injection site were pain, redness (≥ 3 cm), and swelling/induration, and the solicited systemic reactions (considered to be related to the vaccine by the investigator) were fever, axillary temperature ≥ 37.5° C, myalgia/arthralgia, headache, and gastrointestinal tract disorders, such as nausea, vomiting, diarrhea, or abdominal pain.

Statistical methods

A descriptive analysis of safety and immunogenicity was performed in this study. Immunogenicity results were expressed in terms of geometric mean titer (GMT) values and seroconversion rates, with their 95% confidence intervals (95% CI), at each sampling time. The geometric mean of post/pre-booster antibody concentration ratio was also determined. Safety data were described in terms of the number and percentage of subjects with one or more local reactions at the injection site, and the number and percentage of subjects presenting with one or more systemic reactions after each injection.

Ethical considerations

All 119 subjects recruited for this study gave their written informed consent, after having been informed of the nature of the trial and its potential risks. This trial was conducted in accordance with the latest revision of the Declaration of Helsinki, with recommendations of the International Conference on Harmonization (ICH) and Good Clinical Practices (GCP), and with local regulatory requirements. The Ethics Committee of the Ministry of Public Health, Thailand approved the protocol prior to study initiation.

RESULTS

Eighty subjects were included in this study, performed between September 1996 and June 1997. All subjects were considered HAV seronegative at inclusion, based on the qualitative results provided by the ELISA assay performed during the pre-inclusion visit, and received the first vaccine dose. However, quantitative anti-HA antibody titration by modified RIA subsequently showed that two vaccinated subjects had been HAV seropositive (≥ 20 mIU/ml) before immunization with baseline titers of 32 and 56 mIU/ml. These two subjects were kept in the safety analysis, but were excluded from the immunogenicity analysis. The population of 80 vaccinees was equally divided between men and women, 40 females and 40 males; their mean age (SD was 20.8 ± 1.4 years, ranging from 16 to 25 years.

Seventy-nine subjects completed the study; one subject withdrew from the study at the booster visit due to contra-indications to booster vaccination (hepatosplenomegaly due to infectious mononucleosis).

The safety analysis was performed after the first injection on all 80 subjects (n = 80), whereas 79 subjects were evaluated after the booster injection. No serious adverse event occurred during the study. The number and percentage of subjects experiencing one or more reactions after each injection are summarized in Table 1. No immediate reactions were observed after the first dose or after the booster injection. Local reactions (mostly consisting of pain) were observed in 34 (42.5%) subjects after the first injection and in 19 (24.1%) subjects after the booster injection. All reactions subsided in no more

Table 1

Reactogenicity in young adults after primary intramuscular vaccination with PMC inactivated hepatitis A vaccine followed by a booster 24 weeks later (number and percentages of subjects experiencing at least one immediate, local and systemic reaction).

Type of events	First injection n = 80	Booster injection n = 79
Immediate reaction (15 minutes after injection)	0 (0%)	0 (0%)
Local reaction (7 days after injection)	34 (42.5%)	19 (24.1%)
Pain	33 (41.3%)	19 (24.1%)
Induration	1 (1.2%)	0 (0%)
Systemic reaction (7 days after injection)	28 (35.0%)	7 (8.9%)
Fever (Axillary temperature ≥ 37.5°C)	1 (1.3%)	0 (0%)
Asthenia	14 (17.5%)	4 (5.1%)
Headache	6 (7.5%)	1 (1.3%)
GITD	3 (3.8%)	0 (0%)
Myalgia/arthralgia	16 (20.0%)	6 (7.6%)

GITD: Gastro-Intestinal tract disorders.

Table 2

Descriptive seroconversion (SC) rates and geometric mean titer (GMT) values of anti-HAV antibodies in initially HAV seronegative young adults after primary intramuscular vaccination with PMC inactivated hepatitis A vaccine followed by a booster 24 weeks later.

	Primary Immunization		Booster	
	Before vaccination	Day 28	Week 24	Week 28
No.	78	78	77	77
GMT (mIU/ml)	5.1	81.9	140	2,964
95% CI	4.9-5.2	74.4-90.2	116-168	2,467-3,560
Concentration min-max	5-12	27-507	30-978	367-14,592
GMTR (Post-booster/ pre-booster)				21.2
95% CI				17.6-25.6
% SC (antibody titer ≥ 20 mIU/ml)	-	100	100	100
95% CI	-	95.4-100.0	95.3-100.0	95.3-100.0

95% CI = 95% confidence interval.
GMTR = Geometric mean of post/pre-booster antibody titer ratio.

than three days, with the exception of one individual who experienced pain at the injection site for four days. Systemic reactions were observed in 28 (35.0%) subjects after the first injection and in 7 (8.9%) after the booster dose. Most of these reactions were asthenia, myalgia/arthralgia and headache. No systemic reaction lasted any longer than three days, with the exception of two reactions (myalgia lasting 5 days, asthenia lasting 7 days). The incidence of reactions (local and systemic) tended to be lower after the booster than after the primary dose.

Seventy-eight subjects who had no anti-HAV antibodies detectable by means of modified RIA before the first immunization were included in the immunogenicity analysis. GMT values and seroconversion rates are presented in Table 2. All subjects initially seronegative for anti-HAV antibodies reached the seroconversion level of HAV antibody (≥ 20 mIU/ml) one month after receiving the first dose of vaccine that was maintained up to the administration of the booster dose. GMT values were 81.9 mIU/ml 28 days after the first dose and 140 mIU/ml just before booster, *ie* 24 weeks after the first dose. The booster dose induced a striking increase in antibody levels (21-fold increase from the pre-booster antibody concentration) and the post-booster GMT value was 2,964 mIU/ml.

DISCUSSION

The safety and immunogenicity of a new inactivated hepatitis A vaccine produced by PMC were investigated in a Thai population of young adults.

No immediate reaction or serious adverse event was observed during the entire study. Local reactions after the first injection and the booster mainly consisted of transient pain at the injection site. The systemic reactions were principally asthenia and myalgia, as has been previously described with this vaccine (Boutin *et al,* 1996 ; Goilav *et al,* 1995 ; Vidor *et al,* 1996). Similar manifestations and incidences of local and systemic reactions have been reported with other inactivated hepatitis A vaccines (Horng *et al,* 1993; Westblom *et al,* 1994). Reaction rates after the booster dose tended to be lower than those after the primary dose, as has been previously demonstrated for this vaccine (Boutin *et al,* 1996), which indicates that the vaccine does not induce hypersensitization.

Clinical trials conducted in order to determine the protective efficacy of several hepatitis A vaccines (Werzberger *et al,* 1992, Innis *et al,* 1994) have unequivocally demonstrated a high protection rate for each of them. No efficacy trial has been conducted with the PMC vaccine used in this study but there are compelling arguments in favor of its protective efficacy – the different inactivated hepatitis A vaccines used similar strains, are produced on the same cell line, purified by means of similar techniques, and are formaldehyde-inactivated. Antibody levels observed after the first dose of PMC vaccine were similar to those obtained with other hepatitis A vaccines (Goilav *et al,* 1995; Zuckerman *et al,* 1997), and are higher than those observed after human immunoglobulin administration (Zaaijer *et al,* 1993), which has been shown to protect against infection. Based on these data, the PMC hepatitis A vaccine can be expected to provide comparable protective efficacy to the other vaccines tested. It is of note that in the present study, anti-HAV antibody concentrations remained above the seroconversion level of 20 mIU/ml in all immunized subjects until the booster dose was given, 24 weeks later.

The high GMT values obtained after booster vaccination support the expectation that this vaccine would provide long-term protection against infection. Another inactivated hepatitis A vaccine was estimated to provide protection for up to 10 years, which was subsequently confirmed by GMT values observed for up to 6 years after the first vaccination (Maiwald *et al,* 1997). In a comparative study with this reference vaccine, the group of subjects who received the PMC inactivated vaccine were shown to maintain a higher GMT value of anti-HAV antibody two years after vaccination, with equivalent kinetics of antibody decay (Goilav *et al,* 1997). These observations suggest that the PMC vaccine can be expected to provide at least a similar long-term protection to the reference vaccine, *ie* up to 10 years.

The high immunogenicity, as well the good safety profile, of the PMC inactivated hepatitis A vaccine indicates that it is suitable for immunization and will provide long-term protection. This vaccine can be given to prevent hepatitis A in non-immune subjects, especially in areas of intermediate endemicity, where most adults are at risk of contracting hepatitis A infection. Pharmacoeconomic studies would be of value to determine whether inclusion of this vaccine in national preventative

programs would provide a favorable cost-benefit ratio.

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