IMMUNOGENICITY AND SAFETY OF AN INACTIVATED HEPATITIS A VACCINE IN TAIWANESE ADULTS AND CHILDREN

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Abstract. The safety and immunogenicity of an inactivated hepatitis A vaccine (AVAXIM™, 160 antigen units) was evaluated in 190 subjects: 50 children aged from 2 to 5 years, 70 children aged from 6 to 17 years and 70 adults aged from 18 to 30 years in a monocentric, open, non-controlled, phase III trial conducted in Taipei, Taiwan from December 1996 to October 1997. The vaccine was administered intramuscularly, with a two-dose schedule 6 months apart. Clinical adverse events were monitored during the seven days following each injection. Hepatitis A virus (HAV) antibody titers were measured by modified radioimmunoassay on the day of inclusion and four weeks after both the first dose and booster injection.

Among the 190 subjects who received the first dose, 174 (91.6%) were initially HAV seronegative and 16 (8.4%) were HAV seropositive at inclusion. One hundred and seventy-four subjects (91.6%) received the booster dose and completed the study. One month after the first dose, all the subjects, whatever the age, presented HAV antibody titers over 20 mIU/ml. In children (2 to 17 years), the GMT was 136 mIU/ml at week 4 and 7,906 mIU/ml four weeks after the booster dose. In adults (≥18 years), GMT values were 93 mIU/ml at week 4 and 3,655 mIU/ml four weeks after the booster. These results show a strong anamnestic response to the second dose of vaccine and are compatible with long-term antibody persistence in each age group. The vaccine was safe and well tolerated. No vaccine-related serious adverse event occurred. No immediate reaction occurred. The majority of the reactions were reported by adults after the primary injection. Local reactions (pain and redness) were reported by 9.0% and 4.0% of the subjects after the primary and the booster doses, respectively. Systemic reactions (mainly myalgia/arthralgia or asthenia) affected less than 10% of the subjects after the first dose and less than 3% after the booster. Results from this study in a Taiwanese population are consistent with those obtained with the same vaccine in previous European studies in children and adults, and suggest that AVAXIM™ (160 AU) is suitable for use in all subjects aged over 2 years.

INTRODUCTION

Hepatitis A is a vaccine-preventable, enterically transmitted infectious disease that is endemic in many Asian countries. The severity of the disease depends on the age of the patient, being much more severe in adults or adolescents than in children. The prevalence of hepatitis A virus (HAV) infection is closely related to the level of socio-economic development of the country or region. Because of improvements in living conditions in many developing countries, paradoxically, hepatitis A has become a public health problem in these areas, since the incidence of the infection during childhood has been declining, while that of symptomatic HAV infection among adults has been rising (Wu *et al*, 1993; Poovorawan *et al*, 1997a, b; Kunasol *et al*, 1998). Moreover, in recent years several authors have suggested that the prognosis of hepatitis A is worse in the elderly (Forbes, 1988) and in patients with chronic liver diseases, especially hepatitis B and C (Vento *et al*, 1998; Keefe *et al*, 1998). This reinforces the benefit of preventing hepatitis A infection in countries where hepatitis B is endemic.

Children have been identified as a significant reservoir of HAV transmission and must be considered as primary targets of any immunization strategy aiming to reduce the overall incidence of the disease (Margolis and Shapiro 1993; Brewer *et al*, 1995; WHO, 1995; Koff, 1999). Various inactivated, whole-virus hepatitis A vaccines have been developed by different vaccine manufacturers, and those tested have demonstrated clinical

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protection in prospective vaccine efficacy trials (Innis *et al*, 1994; Werzberger *et al*, 1992; Richtmann *et al*, 1996; Mayorga *et al*, 1997) and intervention studies during community-wide outbreaks (Prikazsky *et al*, 1994; McMahon *et al*, 1996; Averhof *et al*, 1996; Werzberger *et al*, 1998; Craig *et al*, 1998). Among the licensed inactivated hepatitis A vaccines, certain ones have been developed with two formulations according to the population targeted: children over two years of age or adolescents and adults. The safety and immunogenicity of the different dosages have been previously reviewed in adults and in children (Nalin *et al*, 1993; Clemens *et al*, 1995; Lea and Balfour, 1997; Horng *et al*, 1993a, b).

An inactivated hepatitis A vaccine, AVAXIM™ containing 160 antigen units (in-house ELISA), manufactured by Pasteur Mérieux Connaught (PMC) (Lyon, France) has been shown to have good immunogenicity and safety in several clinical trials in adults and adolescents (Garin *et al*, 1995; Fisch *et al*, 1996; Goilav *et al*, 1995), and its biological activity as well as the long term persistence of vaccine-induced anti-HAV antibodies have been proven (Vidor *et al*, 1996a,b; Goilav *et al*, 1997). Taiwan is an area of low hepatitis A endemicity (Tsen *et al*, 1991) where the vaccine should be indicated for both children and adults. The aim of the present study was to evaluate the safety and immunogenicity of this vaccine, administered intramuscularly as one primary injection followed by a booster 6 months later, in HAV-seronegative healthy Taiwanese children and young adults aged from 2 to 30 years.

MATERIALS AND METHODS

This was a monocentric, open, non-controlled, phase III trial carried out in Taipei, Taiwan. It was planned to recruit 190 subjects, from three age groups: 50 children aged from 2 to 5 years, 70 children aged from 6 to 17 years and 70 adults aged from 18 to 30 years. Subjects were selected from medical students and children attending primary and high schools in the Taipei district during the period December 1996 to October 1997. Parents or subjects signed an informed consent sheet prior to enrolment. The protocol was approved by the Ethics Committee of the National Taiwan University Hospital before trial initiation, and the study was conducted in according with the Declaration of Helsinki (1987 revision, Hong Kong), and Good Clinical Practice.

A screened subject was excluded from enrolment for any of the following reasons: febrile illness (axillary temperature (≥37.5ºC) or hepatosplenomegaly on the day of inclusion; any previous history of uncontrolled coagulopathy or icterus (excluding neonatal icterus) or treatment with an extracted growth hormone; any known immunological deficiency or treatment with immunosuppressive therapy (including corticosteroids); previous administration of immunoglobulin therapy or another blood-derived product during the six months prior to vaccination or plans to receive such treatment in the next seven months; known allergy to any vaccine component; or previous vaccination against hepatitis A. Pregnant or breast feeding women, or those planning pregnancy during the study period, were also excluded from the study.

Included subjects were to receive two doses of inactivated hepatitis A vaccine given 6 months apart. Blood samples were collected by venipuncture before the first dose of vaccine (for HAV serological status determination), at week 4 (28 days after the first dose) and at week 28 (28 days after the booster dose). Antibody concentrations were determined using a commercial radio-immunoassay (RIA) kit (HAVAB®, Abbott Laboratories, North Chicago, Illinois, USA), modified in order to increase its sensitivity, enabling a lower limit of detection of 10 mIU/ml. This test has previously been used in clinical trials and the results were converted into international units by comparing them with a reference curve generated using the WHO Reference HAV globulin. The antibody levels were expressed in mIU/ml. All the serological titrations were carried out on blinded samples by an independent laboratory (BARC Laboratories, Gent, Belgium).

No screening for HAV antibodies was performed during enrolment, but it was stipulated that should a subject be subsequently found to have been seropositive at inclusion, the second dose of the vaccine was not to be administered.

The vaccine (AVAXIM™, PMC, Lyon, France) is prepared from cultured, purified and formaldehyde-inactivated hepatitis A virus (GBM strain). Each dose contains 160 antigen units (in-house ELISA assay with in-house reference material), 0.3 mg aluminum hydroxide, 2.5 µl phenoxyethanol, 12.5 µg formaldehyde, and up to 0.5 ml of medium 199 and water for injection. For the present study,

the vaccine (batch no. S3139) was presented in 0.5 ml pre-filled syringes mounted with a 16 mm, 25 gauge needle. Injections were performed intramuscularly into the deltoid muscle.

Subjects were monitored for 30 minutes after each injection for the occurrence of any immediate reactions. Solicited and unsolicited local and systemic symptoms occurring over the seven days following each injection were collected using standardized checklists on diary cards; subjects were contacted by telephone on day 3 and day 7 to make sure that the self-monitoring was being performed correctly. Safety data were collected by the investigator during a medical interview at the follow up visits. Results were expressed as the number and percentage of subjects who experienced at least one reaction separately for each injection. Results were described for all subjects, as well as by age group. No statistical between-group comparisons were performed.

The primary objective of the trial was to describe the immunogenicity of the vaccine, by means of seroconversion rates and anti-HAV geometric mean titer (GMT) values, in each age group and in the whole population four weeks after each dose of the vaccine (week 4 and week 28). Data were analyzed in initially seronegative subjects only. No statistical between-group comparisons were performed. Seroconversion was defined as a rise in HAV antibody titers from < 20 mIU/ml before vaccination to ≥ 20 mIU/ml thereafter. Assuming an anticipated dropout rate of 10% of subjects and a percentage of seropositive subjects of 33% in the ages groups (6-17 years) and (18-30 years), it was calculated that 50 children should be included in the youngest age group and 70 subjects in each of the two other age groups. Data were analyzed with SAS software version 6.12 (SAS Inc, Cary, NC).

RESULTS

As planned, a total of 190 subjects were included in the study and were divided into three study groups according to age: 50 children aged from 2 to 5 years, 70 children aged from 6 to 17 years and 70 adults aged from 18 to 30 years. The mean age in years and female/male sex ratios for the three groups are shown in Table 1. Serological analysis at entry revealed that 174 (91.6%) subjects were initially HAV seronegative, while 16 (8.4%) were HAV seropositive. The serological status in each age group is shown in Table 1.

All 190 subjects received the first dose of the vaccine. One hundred and seventy-four subjects (91.6%), of whom 171 were initially HAV seronegative and 3 were borderline HAV seropositive at inclusion, received the booster dose and completed the study. Sixteen subjects withdrew from the study: two subjects refused to continue after the first visit; twelve HAV seropositive subjects did not receive the booster dose because of their serological status; one subject refused to receive the booster injection and did not come back for the third visit, and another subject withdrew from the booster since she had become pregnant after the previous

Table 1
Demographic details and initial hepatitis A antibody status of 190 children and adults enrolled in the study in Taiwan.

Number of subjects included:		(2-5 y) N = 50	(6-17 y) N = 70	(18-30 y) N = 70	All N = 190
Sex					
Male	n (%)	27 (54.0%)	26 (37.1%)	27 (38.6%)	80 (42.1%)
Female	n (%)	23 (46.0%)	44 (62.9%)	43 (61.4%)	110 (57.9%)
Sex ratio F/M		0.85	1.69	1.59	1.38
Age (years)					
Mean age ± SD		3.5 ± 1.0	8.5 ± 2.6	22.5 ± 2.4	12.3 ± 8.3
(Min; max)		(2.0; 5.0)	(6.0; 16.0)	(18.0; 29.0)	(2.0; 29.0)
Serological status					
Positive (≥20 mIU/ml)	n (%)	2 (4.0%)	1 (1.4%)	13 (18.6%)	16 (8.4%)
(20-200 mIU/ml)	n (%)	1 (2.0%)	1 (1.4%)	1 (1.4%)	3 (1.6%)
>200 mIU/ml	n (%)	1 (2.0%)	0	12 (17.1%)	13 (6.8%)

Table 2

Immunogenicity of an inactivated hepatitis A vaccine, AVAXIM™ in children and adults after intramuscular vaccination with two doses given at an interval of six months. Results are presented descriptively as seroconversion rates (SC, %) and Geometric Mean Titers (GMT, mIU/ml) of anti-HAV antibodies, in initially HAV seronegative subjects according to age groups (full set analysis).

Age group (years)		Day 0	Week 4	Week 28
2-5	Number of subjects	48	48	48
	GMT	5.6	106.8	8174
	Titer range (min; max)	(5; 15)	(56; 474)	(1439; 26736)
	[95% CI]	[5.1; 6.1]	[91.6; 124.5]	[6934; 9635]
	% SC	0%	100%	100%
	[95% CI]		[92.6; 100]	[92.6; 100]
6-17	Number of subjects	69	68	68
	GMT	5.5	161.7	7722
	Titer range (min; max)	(5; 18)	(59; 894)	(1404; 49187)
	[95% CI]	[5.2; 5.9]	[135.6; 192.8]	[6658; 8957]
	% SC	0%	100%	100%
	[95% CI]		[94.7; 100]	[94.7; 100]
2-17	Number of subjects	117	116	116
	GMT	5.5	136.2	7905.9
	Titer range (min; max)	(5; 18)	(56; 894)	(1404; 49187)
	[95% CI]	[5.3; 5.9]	[120.2; 154.4]	[7090; 8816]
	% SC	0%	100%	100%
	[95% CI]		[96.9; 100]	[96.9; 100]
18-30	Number of subjects	57	57	55
	GMT	8.6	92.8	3655
	Titer range (min; max)	(5; 19)	(29; 514)	(426; 26016)
	[95% CI]	[7.5; 9.8]	[80.1; 107.4]	[2879; 4641]
	% SC	0%	100%	100%
	[95% CI]		[93.7; 100]	[93.5; 100]
2-30	Number of subjects	174	173	171
	GMT	6.4	120.0	6169
	Titer range (min; max)	(5; 19)	(29; 894)	(426; 49187)
	[95% CI]	[6.0; 6.8]	[108.6; 132.6]	[5480; 6944]
	% SC	0%	100%	100%
	[95% CI]		[97.9; 100]	[97.9; 100]

visit. Three subjects shown to be HAV seropositive at inclusion, but whose initial HAV antibody titer was close to the threshold of 20 mIU/ml (≤50 mIU/ml), received a booster injection since their immunity against HAV could not be absolutely established. Nevertheless, these subjects were excluded from the analysis of immunogenicity.

Overall immunogenicity data are shown in Table 2. Four weeks after the first dose of the vaccine, all subjects in each age group had seroconverted (≥20 mIU/ml, mRIA HAVAB®; Abbott). In children (subjects aged 2 to 17 years), a strong anamnestic response to the second dose of vaccine was observed, with the post-booster GMT value (7,906 mIU/ml) increasing 58-fold from the value measured four weeks after the first dose (136 mIU/ml). Likewise in adults (subjects ≥18 years), the post-booster GMT (3,655 mIU/ml) increased 39-fold compared to the post-first dose value (93 mIU/ml). In the three subjects whose HAV antibody titers ranged from 20 to 50 mIU/ml at inclusion and who nevertheless received a booster injection, the anti-HAV titers measured at Weeks 4 and 28 showed an immune response to vaccination similar to that observed in initially HAV seronegative subjects.

Reactogenicity of the inactivated hepatitis A vaccine is summarized in Fig 1. No subject

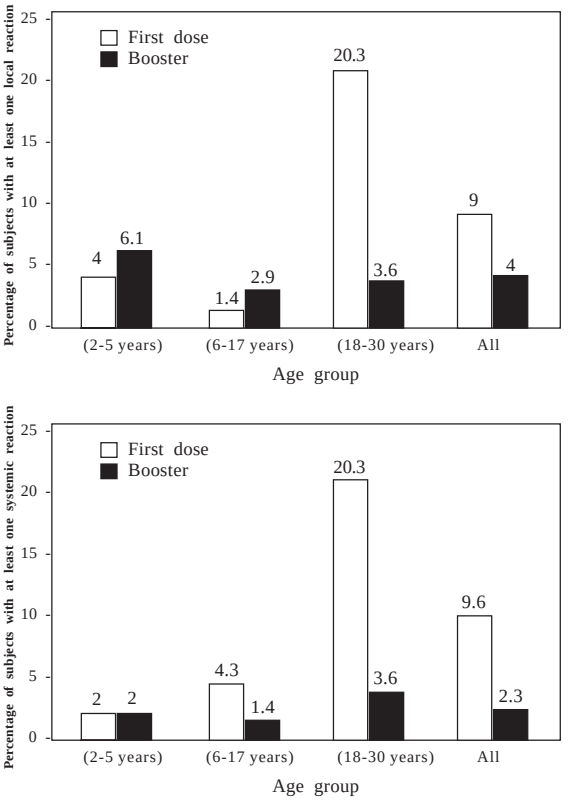

Fig 1–Reactogenicity in children and adults after intramuscular vaccination with two doses of an inactivated hepatitis A vaccine, AVAXIM™, at an interval of six months. Results are shown as number and percentages of subjects experiencing at least one local or systemic reaction.

presented an immediate reaction after any of the two injections. Local reactions, restricted to pain at injection site and redness, were experienced by 9% of subjects after the first dose, mainly in adults, and 4% of all subjects after the booster dose. All local reactions occurred within 2 days after vaccination and were transient. After the first vaccine dose, systemic reactions, of which asthenia, myalgia or arthralgia, gastro-intestinal tract disorders, and headache were the most common symptoms, in 9.6% of subjects. After the booster injection, 2.3% of subjects reported systemic symptoms, of which asthenia was the most common reaction. The symptoms occurred within 2 days of vaccination and never persisted more than 3 days, except for three reactions after the first dose in adult subjects that lasted 7 days (1 case of asthenia, two cases of myalgia/arthralgia). One serious adverse event (hospitalization for pneumonia in a 20-year-old woman) was reported during the trial, but was considered as not related to the vaccination by the investigator.

DISCUSSION

There are different epidemiological patterns of hepatitis A infection that reflect standards of hygiene and sanitation of the population in which the virus spreads (Gust, 1992). The improvements in standards of living conditions worldwide during the last 20 years have led to an overall drop in HAV incidence, and the shift of some countries from highly endemic areas to intermediate endemicity. However, this has also resulted in an epidemiological shift of HAV-prevalence to older age groups, in which disease is more severe. An increasing number of children and young adults are susceptible to the residual circulating HAV especially in intermediate endemicity areas. Studies on HAV antibody prevalence showed a low prevalence in young children living in urban areas of Argentina (Gonzales *et al*, 1996, 1997; Iriart *et al*, 1997) and in children and adolescents in the Philippines (Cabahug, 1992). Likewise, antibody prevalence among children in Thailand has declined during the last ten years (Poovorawan *et al*, 1997a, b).

Prevention strategies may be developed on the basis of hepatitis A epidemiology. Vaccination is not routinely recommended in developing, highly endemic countries, except for travelers. In industrialized regions, vaccination is recommended for individuals in high-risk groups, such as health care workers, day care workers, homosexual men, users of injecting drugs, cooks, sewer maintenance engineers, tourists, etc (CDC, 1996). In countries with an intermediate level of risk, there is concomitantly a high level of HAV infection and a low level of immunity. The introduction of HAV into communities in which the majority of individuals have not been immunized may results in local outbreaks (Koff, 1998). Vaccination programs are likely to be of value in these countries or areas and should be focused on routine childhood vaccination (Brewer, 1995; WHO, 1995; Bell *et al*, 1998; Koff, 1999).

In our study, carried out in Taipei, Taiwan, there was a low seroprevalance of HAV in children and adolescents, with 2/50 (4.0%) children aged 2 to 5 years and 1/70 (1.4%) aged 6 to 17 years being seropositive. In contrast, 13/70 (18.6%) of

young adults (18 to 30 years) were found to have HAV antibodies, presumably who were infected previously during their childhood. These data are consistent with the epidemiological shift of hepatitis A infection to an older population in this region (Wu *et al*, 1993) and demonstrate the increased susceptibility of children and adolescents in Taiwan to hepatitis A infection.

In this study, we investigated the immunogenicity and safety of an inactivated hepatitis A vaccine that can be used for all subjects aged 2 years and above. AVAXIM™ (160 AU) appeared highly immunogenic in children, adolescents and young adults. One month after a single dose of vaccine, 100% of subjects in each age group seroconverted for HAV antibodies. Subjects demonstrated strong anamnestic responses to the second dose of the vaccine, eliciting very high antibody titers and confirming the establishment of immune memory. These results are consistent with those obtained with the same vaccine in healthy adults (Garin *et al*, 1995; Fisch *et al*, 1996; Goilav *et al*, 1995; Vidor *et al*, 1996a, b). Our data in children are comparable to those obtained recently in a study conducted in Venezuela, following the administration of the same inactivated hepatitis A vaccine to children aged 4 to 15 years, in which all subjects seroconverted just 2 weeks after the first vaccine dose, with a GMT of 74 mIU/ml, and showed a strong response to booster given six months later, as evidenced by a GMT of 6,999 mIU/ml four weeks after the second vaccine dose (Castillo de Febres, 2000).

While no between-group statistical comparisons were planned in this study, it appeared that children had a stronger anamnestic response to the booster dose of vaccine than adults. Four weeks after the second vaccine dose, the highest GMT was in the youngest age group (8,174 mIU/ml). The lowest GMT was in the group aged 18 to 30 years (3,655 mIU/ml), and the 95% confidence interval for this value did not overlap those of the younger age groups (Table 2).

While the duration of protection conferred by the high antibody titers elicited by the second vaccine dose remains unknown, according to estimates of the persistence of anti-HAV antibodies derived from kinetic models, protection against HAV infection may possibly last for 15 to 30 years (Van Damme *et al*, 1994; Totos and Papaevangelou, 1994; Maiwald *et al*, 1997; Wiedermann *et al*, 1992; Berger and Just, 1992; Chan *et al*, 1999; Dentico *et al*, 1996; Lee *et al*, 1995); detectable antibody levels can be found in roughly 40% of immunized individuals after 30 years (Wiens *et al*, 1996). Nevertheless, immune memory, which has been demonstrated in naturally immune persons even in the absence of detectable antibody (Villarejos *et al*, 1982), should induce an anamnestic increase in seroneutralizing antibody levels to provide late protection against symptomatic disease in previously immunized persons (Nalin, 1995; Stapelton and Lemon, 1997).

AVAXIM™ (160 AU) appeared safe and well tolerated in all age groups. There was no vaccine-related serious adverse event. No immediate reaction occurred. Very few children experienced reactions after either dose, with the majority of the reactions reported by adults after the primary injection. Side effects of most vaccines tend to be more frequent in adults than in children. Furthermore, this effect may have been augmented by the fact that the adult subjects, who were all medical school students, were of a nature to observe and report even very mild events that would normally go unperceived. After the second dose, the reaction rates were considerably lower, and more consistent with usually reported rates of reactions. Nevertheless, it has been previously noted with the same vaccine that the overall rates of adverse reactions observed in Venezuelan children were lower than those reported in adults (Vidor 1996a, b; Castillo de Febres, 2000).

The data from our study show that AVAXIM™ was safe and immunogenic in a population of children and adults in Taiwan, and suggest that this vaccine would be suitable for use in all subjects of 2 years and above. The availability of a single vaccine that can be given to subjects of all ages would facilitate choice, storage and administration of hepatitis A vaccine.

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