REACTOGENICITY AND IMMUNOGENICITY OF A VARICELLA VACCINE IN HEALTHY SERONEGATIVE AND SEROPOSITIVE SUBJECTS

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Abstract. The epidemiology of varicella appears to be changing: an unexplained upward age shift in varicella prevalence and a subsequent dramatic rise in morbidity and mortality among adolescents and adults have highlighted the importance of effective varicella mass vaccination programs. This age shift is being seen in temperate regions but is particularly marked in tropical and sub-tropical regions. To assess the need for serological pre-screening in mass vaccination programs, we performed an open study to compare the reactogenicity and immunogenicity of a varicella vaccine in initially seronegative and seropositive subjects to see whether there was an increase in reactogenicity among initially seropositive subjects. Two hundred and forty-six seronegative and seropositive male and female subjects, aged 9 months to 60 years, received a single dose of a live attenuated varicella virus (Oka-strain) vaccine, Varilrix™ (GlaxoSmithKline Biologicals, Rixensart, Belgium). Subjects were categorized according to antibody status and age group; serum antibodies were measured before and after vaccination (day 42). The study showed that there was no difference in reactogenicity in initially seropositive vaccinees compared with initially seronegative subjects. The varicella vaccine was found to be safe and well tolerated in all age groups. Ninety-eight percent of initially seropositive and 94.8% of initially seronegative subjects reported no clinical signs or symptoms during the 42-day follow-up period. The vaccine was immunogenic in both groups. The seroconversion rate after 6 weeks in initially seronegative subjects was 94.3%. In 53.0% of initially seropositive subjects of all age classes, a 4-fold rise in antibody titer was observed.

INTRODUCTION

Varicella is a highly communicable disease with a secondary attack rate of up to 90% among household contacts (Straus *et al*, 1988). While varicella is usually a benign disease among healthy children, the incidence of complications increases dramatically during adolescence and adulthood. It is estimated that there are 4 million cases of varicella each year in the United States; in temperate regions such as the United States, almost all individuals will have been infected by the time that they reach adulthood; most acquire the disease in childhood (White, 1997).

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An unexplained change in the epidemiology of varicella: an upward age shift in temperate and particularly tropical and sub-tropical regions highlights the importance of effective varicella mass vaccination programs. For example, in the United Kingdom, the incidence of varicella among those older than 14 years of age has risen steadily during the past 20 years (Miller *et al*, 1993). This trend is reflected in the United States, where the reported incidence of varicella among those aged between 17 and 27 years has increased more than 18-fold since 1975 (Gray *et al*, 1990).

Epidemiology

Two distinct patterns of varicella infection have been described in the literature: in temperate regions, varicella is mainly a childhood disease with a low incidence of complications;

in tropical and subtropical areas however, the proportion of adolescents and adults infected is considerably greater. An early 1990's study in Manila found that the seroprevalence of varicella antibodies increased with age from 30% in those under five years of age to 55% in children aged 6 to 10 years, and to 57% in those aged 11 to 15 years; consecutive increments were found up to 30 years of age and in those over 30 years, a > 92% prevalence was seen (Barzarga *et al*, 1994). In addition, a recent study in Thailand showed that one in three adolescents and young adults lacked natural immunity against varicella (Migasena *et al*, 1997). Seasonal and regional national variations in acute illness in some Southeast Asian countries suggest that temperate climates might favor transmission of the virus as outbreaks occur during cooler months (Wah *et al*, 1994)). The role of environmental factors in the spread of varicella has yet to be clearly understood; however, it is thought that the current migration of non-immune adults from the tropics to North America and Europe increases the significance of varicella infections in these geographical areas (Thomas and Weller, 1995).

The disease is generally mild in children, usually self-limiting, and complications are rare; in adults the varicella-associated mortality rate is 25 times higher than in children due to a higher incidence of complications (Maretic and Cooray, 1963; Sinha, 1976; Preblud *et al*, 1984). Furthermore, in esthetic terms, varicella rashes are usually much more severe in the older age groups, often resulting in permanent scarring (Malik, 1996). A study of facial scarring after varicella involving 250 individuals of younger than one year of age to older than 50 years of age, showed that the highest proportion of those with five or more persisting facial scars was to be found in the 20 - 29 year age group (Jezek *et al*, 1981).

The medical and social burden of varicella morbidity and mortality strengthens the argument for routine universal varicella vaccination in healthy children, adolescents and adults - particularly in Southeast Asia - in order to give protection to against the associated risks of varicella in the older age groups. A cost-benefit analysis in the United States indicated a net reduction in the financial burden faced by society: fewer working days were lost and medical costs were cut - findings that support preventive varicella vaccination in children (Lieu *et al*, 1994). In developing countries, where disease in adulthood is more likely, the social cost of the disease is likely to be even higher than it is in countries in which the disease is mainly encountered during childhood because of a greater adverse impact on work.

Approaches to varicella vaccination vary considerably throughout the world with some countries still favoring vaccination only for immunocompromised patients and their close contacts. However, in the past few years, in most countries of Asia (Hong Kong, Malaysia, Indonesia, the Philippines, Singapore and Thailand) and elsewhere, the trend has been towards the vaccination of healthy children (in the second year of life), adolescents and adults. This trend follows the lead given by Japan and South Korea, the first countries to adopt the vaccination of healthy subjects with the Oka strain vaccine (licensed for general use in healthy children since 1986) (Ramkissoon *et al*, 1995). Both the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Pediatrics (AAP) recommended that varicella vaccination should be given in the second year of life and be extended to young adolescents and adults without a history of the disease or previous vaccination (ACIP,1999). In the USA, the Oka strain varicella vaccine was licensed for routine immunization in March 1995. The vaccination of healthy infants adolescents has been endorsed in Finland where the Public Health Institute recommends that healthy children over 12 months and seronegative adolescents are vaccinated against varicella (National Public Health, in press). This vaccination program has also been approved in other European countries, including Austria, Belgium, Germany, Luxembourg and Sweden.

A new formulation of the live attenuated Oka strain varicella vaccine, Varilrix™,

(GlaxoSmithKline Biologicals), which is heat stable and can be kept for 2 years at 4-8ºC, has been extensively tested in healthy populations for safety and immunogenicity. Such benefits simplify mass vaccination program logistics. The present study was performed both to investigate whether vaccination with Varilrix™ resulted in any differences in reactogenicity between initially seropositive subjects and initially seronegative subjects and to study the immune response in these two groups.

MATERIALS AND METHODS

This open clinical study was approved by the appropriate Ethics Review Committee and was conducted at the College of Public Health, University of the Philippines, Manila. Written informed consent was obtained from all subjects or their parents/guardians before enrolment. The study was conducted in accordance with the Declaration of Helsinki, taking into consideration the European Commission's Good Clinical Practice (GCP) guidelines.

Study design

Two hundred and forty-six healthy subjects (147 initially seronegative and 97 initially seropositive) of between 9 months and 60 years of age participated in the study. The ratio of females to males was 3:2 and the mean age was 12.4 years. To be eligible for the study, subjects had to be declared medically fit after a clinical examination and had to fulfil a set of eligibility criteria; subjects had to be free from allergic or adverse reactions to any previous vaccination and must not have recieved any immunoglobulins or blood products during the 3 months prio to blood sampling or the administration of the study vaccine. Female participants of childbearing age were asked to use an effective method of contraception.

At the time of enrolment, subjects were allocated a subject number and assigned to a study group according to their age and serological status. Immunogenicity was analyzed by indirect immunofluorescence (IIF) for VZV antibodies; an ELISA test kit was employed to determine serostatus and subjects were assigned to 2 groups: initially seronegative and initially seropositive, which were each stratified by age (< 7 years, 7-12 years, ≥ 13 years). On day 0, subjects were vaccinated by intramuscular infection of the right deltoid muscle with a live attenuated Oka strain varicella vaccine (Varilrix™) with a mean titer of $10^{4.1}$ plaque forming units (pfu) per 0.5 ml dose; each subject was given a 0.5 ml dose.

Blood samples were collected for pre- and post-vaccination antibody measurements on the day of vaccination (day 0) and 42 days post-vaccination. Diary cards were used by subjects or their parents/guardians to record symptoms (rashes, skin eruptions) and any severe adverse reactions experienced either on the day of vaccination or during the 42 subsequent days. In the event of any skin rash or eruption, subjects were asked to visit the investigator for clinical diagnosis and, if vesicles were present, for collection of vesicular fluid for varicella virus isolation. Subjects were also asked to contact the investigator if any serious adverse events occurred. On day 42, subjects were seen again by the investigator for a review of adverse events that had occurred during the study period.

Laboratory methods

The serostatus screening was performed using an ELISA test kit (Enzygnost® anti-VZV/IgG, Behringwerke Laboratories, Germany) for varicella-zoster IgG detection. All tests were carried out according to the manufacturer's instructions; subjects with antibody titers below 0.2 absorbance units were deemed as seronegative. The antibody titer of the sample was expressed as a reciprocal of the last dilution that tested positive. Separated blood samples collected for antibody determinations were stored at -20ºC until they were tested at the vaccine manufacturer's laboratory in Rixensart, Belgium. Specific VZV IgG antibodies were determined in both pre-vaccination and post-vaccination serum samples with

a commercial indirect immunofluorescence (IIF) kit, the Virgo® VZV IgG Indirect Immunofluorescent antibody test (Pharmacia).

Analysis of results

Seroconversion was defined as the appearance of specific anti-VZV antibodies in the serum of initially seronegative subjects (titer of < 4 in pre-vaccination to ≥4 in post-vaccination serum). A 4-fold or greater rise in a positive pre-vaccination antibody titer was considered as a booster response in the initially seropositive subjects. Geometric mean titers (GMTs) of specific varicella antibodies were calculated in pre- and post-vaccination sera by taking the anti-log of the mean of the log transformation of positive titers. Confidence intervals of 95% were calculated for all GMTs. In the event of rashes, the number of papules or vesicles presented by a subject were counted. Other clinical signs and symptoms observed during the follow-up period were described by their intensity and their relationship to the vaccine was assessed by the investigator.

Statistical methods

The demographic characteristics (age and sex) of the study groups were tabulated and the mean age, range and the standard deviation were calculated for all males and females separately and together for the seronegative and seropositive populations (established by IIF-I). Seroconversion rates and GMTs, with 95% confidence intervals, of specific VZV antibodies in seroconverters were calculated by defined age groups and for the total study group at day 42 post-vaccination. For seropositive subjects, the booster response rates (at day 42) and the pre- and post-vaccination GMTs, with 95% confidence intervals, were calculated per age group and for the total study group. All analyses were performed using SAS 6.10 with a type I error (α) of 5%.

RESULTS

All 246 subjects enrolled in the study were vaccinated and included in the analysis of vaccine reactogenicity (Table 1). Two hundred and six subjects were analysed for immunogenicity (Table 2), 40 subjects having been excluded for either different forms of protocol violation, including blood sampling outside the range of 42 - 56 days, or insuf-

Table 1
Demographics of subjects enrolled and analysed for reactogenicity.

Serostatus	Sex	No.	Mean age (years)	Min age (years)	Max age (years)	SD
S-	Male	63	9.6	1	60	9.98
	Female	84	8.9	1	22	7.32
	Total	147	9.2	1	60	8.54
S+	Male	33	16.1	3	44	9.82
	Female	64	18.1	3	46	9.60
	Total	97	17.4	3	46	9.67
Unknown	Male	2	4	3	5	1.41
	Total	2	4	3	5	1.41
Total	Male	98	11.7	1	60	10.31
	Female	148	12.9	1	46	9.51
	Total	246	12.4	1	60	9.83

N = total number of subjects; SD = standard deviation; S- = initially seronegative subjects (IIF titer); S+ = initially seropositive subjects (IIF titer).

ficient serum for antibody measurement. Elimination of these subjects did not introduce a bias into the study population analysed for immunogenicity.

Reactogenicity

The vaccine was safe and well tolerated in all age groups. Over 96% of subjects reported no clinical signs or symptoms during the 42-day follow-up period. There were no local reactions to the vaccine in both seropositive and seronegative subjects. General signs and symptoms were elicited from 2% of the seronegative subjects and from 5.2% of the seropositive subjects: the symptoms were skin reactions with a varicella-like rash which occurred in six subjects (three initially seronegative and three initially seropositive) on day 1-23 after vaccination. All these cases had less than 50 papules and all resolved without any sequelae within 11 days of onset. All the rashes were considered by the investigator to be either related or possibly related to vaccination. Two other subjects (initially seropositive) reported moderate fever (38ºC and 39ºC) lasting three days and two days respectively. Both cases were considered by the investigator to be possibly related to the vaccination. No serious adverse events were reported in this study.

Table 2
Subjects analysed for overall immunogenicity.

Age group (years)	No. of S+	No. of S-	Total
< 7	7	59	
7 - 12	24	28	
> 13	52	36	
Total	83	123	206

S+ = Subjects seropositive for anti-VZV at pre-vaccination (titer by IIF).
S- = Subjects seronegative for anti-VZV at pre-vaccination (titer by IIF).

Immunogenicity

The overall seroconversion rate at 42 days after vaccination for initially seronegative subjects was 94.3% (116/123) with a GMT of 44.2 (Table 3). Fifty-three percent of subjects, initially seropositive, showed a booster response

Table 3
Seroconversion rates (%) and geometric mean titers (GMTs) of antibodies against varicella zoster virus for initially seronegative subjects.

Age group	Timing	No.	Seroconversion		Antibody titer	
			S	%	GMT	GMT 95% CI
< 7 yrs	Pre	59				
	PI d 42	59	57	96.6	50.8	38.6,66.9
7-12 yrs	Pre	28				
	PI d 42	28	28	100.0	50.0	31.9,78.3
≥13 yrs	Pre	36				
	PI d 42	36	31	86.1	30.6	19.9,47.1
Total	Pre	123				
	Post	123	116	94.3	44.2	38.5,54.5

N = number of subjects vaccinated in each age group.
S = number of subjects that seroconverted.
Pre= pre-vaccination.
PI d42 = post-vaccination day 42.

Table 4
GMTs of antibodies against varicella zoster virus and booster response for initially seropositive subjects.

Age group	Timing	No.	S	% S+ ≥4-fold- booster response	GMT	GMT 95% CI
< 7 yrs	Pre	7			344.6	135.8 - 873.9
	PI d 42	7	4	57.1	1,378.2	920.1 - 2,064.3
7 - 12 yrs	Pre	24			263.5	144.1 - 481.9
	PI d 42	24	11	45.8	1,024.0	699.5 - 1,566.2
≥ 13 yrs	Pre	52			252.6	183.1 - 348.6
	PI d 42	52	29	55.8	838.4	648.6 - 1,083.8
Total	Pre	83			262.5	199.4 - 345.6
	Post	83	44	53.0	926.4	754.0 - 1,138

Pre = pre-vaccination; PI d 42 = post-vaccination day 42; S = number of subjects showing a booster response.

with a GMT of 926.4 (Table 4). The booster response rates among the subjects who were initially seropositive were 57.1% for the <7-year age group, 45.8% for the 7-12 years group and 55.8% for the ≥13 years group. Only one subject (a 7 year-old) showed a decrease in titer. The under-7-years age group showed the largest increase in GMT, by a factor of 4.4.

DISCUSSION

The reported pattern of age prevalence of varicella has shifted toward the older age groups in recent years: this upward shift in the age-specific incidence of varicella infection has wider implications for future morbidity and mortality.

Varicella in adolescents and adults has a major economic impact resulting from physician visits, hospitalizations due to clinical complications and lost work time (Leventon *et al*, 1978; Guess *et al*, 1986; Lieu *et al*, 1994; Ramkissoon *et al*, 1995; ACIP, 1999).

The risks associated with contracting varicella in adolescence and adulthood highlight the need for safe, effective vaccines for mass vaccination programs to protect children, non-immune-adolescents and adults. In tropical regions the shift in incidence of varicella towards older age groups is more pronounced. However, coverage of this age range is best achieved by vaccinating infants in conjunction with other childhood vaccines. Many countries in Southeast Asia vaccinate with 1 dose of varicella vaccine between 12 months and 12 years, while 2 doses are recommended for individuals from 13 years of age. Antibody response studies after 1 dose of vaccine have shown that the average seroconversion rate in children aged 12 months to 12 years is 97%. In adolescents and adults over 13 years of age, 86% seroconverted after the first dose of vaccine, and 99% seroconverted after a second dose given 4 to 8 weeks later. In other studies, with subjects older than 13 years of age, the proportion of vaccine recipients who seroconverted did not differ by age (Arbeter *et al*, 1986; Kuter *et al*, 1991; White *et al*, 1991).

With surveillance data indicating an upward shift in the age distribution of varicella, the data from this study is reassuring, showing that live attenuated varicella vaccine (Oka strain) is safe and well tolerated in both the individuals seronegative and seropositive to VZV. The safety profile of the live attenuated varicella vaccine Oka strain and its immunogenicity

have been evaluated in controlled clinical trials involving 10,000 subjects of all ages (Ramkissoon *et al*, 1995). The Oka strain has been shown to be attenuated and stable (André, 1985; Tsolia *et al*, 1990).

This study evaluated the reactogenicity and immunogenicity of a single dose of a live attenuated varicella virus vaccine, Varilrix™, in healthy seronegative and seropositive subjects of different ages. In general, the reactogenicity and immunogenicity data from this study compare very well with the results of other Oka strain varicella vaccine studies and show that this refrigerator-temperature-stable vaccine was well tolerated in both seropositive and seronegative subjects. Varilrix™ proved to be immunogenic in both seropositive and seronegative subjects of all ages and gave a 4-fold booster response in >50% of initially seropositive individuals.

During the 42-day follow-up period 96.7% (238/246) of subjects reported no clinical signs or symptoms, which is in line with the results of a series of prospective studies with Varilrix™ in seven countries involving 1,576 (VZV-seronegative) children (Tan *et al*, 1996). The vaccine used was well tolerated with no local reactions reported.

Varicella-like rashes were seen in both initially seronegative and initially seropositive subjects (with titers of > 128), suggesting that not all varicella-like rashes are linked to varicella infection. The rashes were maculopapular, few in number and disappeared in 3-5 days. The rashes could be linked to other circulating viral infections and not be influenced by varicella vaccination. The typical incidence of varicella-like breakthrough rashes is around 4% per year with a median of 5-10 rashes following primary vaccination with varicella vaccines (LaRussa *et al*, 1997).

The vaccine was highly immunogenic in the two groups analysed. In the initially seronegative subjects, the most vigorous response (97% - 100% seroconversion) was seen mainly in younger subjects (≤12 years of age). The lower seroconversion rate (86%) observed in subjects older than 13 years suggests that there is a need for a 2-dose schedule in this age range. In initially seropositive subjects, the booster response rates in older subjects were similar to those in the young. Post-vaccination GMTs were higher in younger subjects and were lower in the older age groups in both initially seronegative and initially seropositive. The antibody response seen in the younger age groups reflects that seen in healthy children who had received single dose varicella vaccinations in other studies. In a recent review of a series of studies performed with Varilrix™ (Tan *et al*, 1996), as well as in a review by Gershon *et al* (1992), seroconversion rates in healthy children after a single dose were generally ≥95%.

This study demonstrates that this vaccine is equally well tolerated in seronegative and seropositive individuals of all ages. The elimination of serological pre-screening will limit the cost of mass vaccination programs and make it easier to vaccinate adolescents and adults who are often more difficult to reach than young school-age children who are covered by national programs. Simplifying mass vaccination programs by the elimination of pre-screening will have an important impact on vaccine coverage and compliance. A routine varicella vaccination program for healthy children will prevent an estimated 94% of all potential cases of varicella, provided that the vaccination coverage rate is 97% at school entry (Lieu *et al*, 1994).

A simple and practical approach to mass varicella vaccination would be to integrate varicella vaccination into the National Expanded Programs of Immunization for children as well as extending vaccination to adolescents and adults who have no a history of the disease or varicella vaccination. The age at which vaccination is given could be selected to reflect local epidemiology and practices and to ensure the broadest coverage possible, thereby reducing the circulation of wild virus to a minimum.

The Advisory Committee on Immunization Practices (ACIP) (1999) recommends a

routine single dose for all children between 12 - 18 months of age and co-administration with measles, mumps and rubella (MMR) vaccination. There is data to show that the concomitant but separate-site administration of varicella and MMR vaccines gives good seroconversion rates and is logistically more efficient (Brunell *et al*, 1988; Watson *et al*, 1996). Mass varicella vaccination should be backed by effective post-licensure studies and surveillance, as well as by epidemiological data in order to evaluate the persistence of immunity and the need for booster strategies.

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