REACTOGENICITY AND IMMUNOGENICITY OF A LIVE-ATTENUATED REFRIGERATOR-STABLE VARICELLA VACCINE (OKA STRAIN) IN HEALTHY SERONEGATIVE SUBJECTS AGE 10 MONTHS TO 12 YEARS

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Abstract. This study assessed the immunogenicity and reactogenicity of a live-attenuated varicella vaccine (Oka strain), *Varilrix*™ in Indonesian children age 10 months to 12 years. A total of 300 seronegative subjects were stratified into three age subgroups (10 months to < 3 years, 3 years to < 7 years and 7 to 12 years) and all received a single-dose of Oka strain varicella vaccine. One solicited local symptom (injection site soreness) was reported during the 43-day post-vaccination follow-up period. Fever (29/295; 10%) was more prevalent than rash (3/295; 1%) but the incidence of grade 3 fever (defined as axillary temperature of >39ºC) was infrequent. No grade 3 unsolicited events and no serious adverse events were reported. The vaccine proved to be immunogenic in all age groups; all but one subject seroconverted for anti-varicella antibodies 43-days post-vaccination. This study demonstrated that the live-attenuated varicella vaccine (Oka strain) was well tolerated and immunogenic with no safety issues when administered as a single dose primary vaccination to healthy, seronegative Indonesian subjects age 10 months to 12 years.

INTRODUCTION

Varicella zoster virus Vericella zoster (VZV) primarily causes chickenpox, an extremely communicable disease which is characterized by vesicular rash accompanied by fever (Kreth *et al*, 2008). This infection is considered a mild disease in healthy children, but it has the potential to be fatal. It can also result in complications such as pneumonia, encephalitis and bacterial super infection of the skin lesions in young children as well as adolescents, adults and immunocompromised children (Sloan and Burlison, 1992; Miller *et al*, 1993; Meurice *et al*, 1996; Diaz-Mitoma *et al*, 2000; Diaz *et al*, 2006).

The epidemiological patterns of varicella infection vary across different climatic regions. In temperate countries, such as the UK and US, most cases of varicella are seen in children <10 years old, and the majority of the population has seroconverted by adolescence. However, an upward age shift in the epidemiology of varicella had been seen in tropical regions of South and Southeast Asia, such as India, Thailand, Singapore and the Philippines. This epidemiological change stresses the need for effective varicella mass vaccination, since the disease tends to be more severe in adolescents and adults (Tan *et al*, 1996; Barzaga *et al*, 2002).

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Seroprevalence data in Southeast Asia have shown that there is a low seroprevalence among children <5 years old and >90% seroconversion is seen in those ≥30 years of age. This trend leads to low herd immunity in children and young adults, making them susceptible to varicella infection at an older age (Lee, 1998; Barzaga *et al*, 2002). The occurrence of this disease is almost universal; an estimated 60 million cases occur worldwide each year, of which 4 million cases are reported in the US alone (Barzaga *et al*, 2002; Lichenstein, 2006).

A single parental strain of wild type VZV (Oka strain) was originally isolated in Japan in 1971 and has been used for the development of all live- attenuated varicella vaccines currently marketed in many countries (Lau *et al*, 2002). This vaccine was first licensed for use in immunocompromised children in 1984 and healthy children in Germany and Sweden in 1994 (Clements, 2000; Chiu and Lau, 2005). In the United States, since the introduction of universal mass vaccination (UMV) in 1995, the annual varicella disease incidence has reduced by 70% (Chiu and Lau, 2005). Although, a single dose of varicella vaccine was found to decrease incidence of the disease considerably, the Advisory Committee on Immunization Practices (ACIP), in 2005 recommended the use of a second vaccine dose in the USA in outbreak settings (Lopez *et al*, 2006). Germany also introduced UMV against varicella in 2004 (de Moira and Nardone, 2005, Kreth *et al*, 2008).

Initial formulations of the Oka strain varicella vaccine (frozen formulation) required storage at -20ºC (Chiu and Lau, 2005). Considering the problems associated with production and cold chain management with a frozen vaccine, a reformulated vaccine was developed (Kreth *et al*, 2008).

This reformulated Oka strain, refrigerator-stable vaccine, was prepared without modifying the viral strain that can be stored at 2-8ºC with a shelf-life of two years (Meurice *et al*, 1996; Diaz *et al*, 2006). Currently, the reformulated vaccine is licensed in 92 and launched in 89 countries worldwide (Kreth *et al*, 2008). The ACIP currently recommends implementation of a routine 2-dose varicella vaccination program for children, with the first dose to be given at 12-15 months of age and the second dose at 4-6 years of age. A catch-up dose of the vaccine is to be administered to children, adolescents and adults if they had received a first vaccine dose previously and routine vaccination of all healthy persons ≥13 years old with no evidence of immunity (Marin *et al*, 2007).

The primary objective of this clinical study was to assess the reactogenicity and safety of the reformulated live-attenuated varicella vaccine (Oka strain) in healthy seronegative Indonesian subjects age between 10 months and 12 years old. The immunogenicity of the vaccine was also assessed in this study.

MATERIALS AND METHODS

Subjects and ethics

This open, self-contained clinical study was approved by the appropriate Ethics Review Committee and was conducted between May 1998 and October 1998 at the Ciptomangunkusumo General Hospital, Jakarta, Indonesia. Written informed consent was obtained from parents/guardians of all subjects at the time of screening. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines.

In this study, 300 healthy, seronegative children age 10 months to 12 years old were enrolled. The subjects were stratified into three subgroups based on age:10 months to <3 years, 3 years to <7 years, 7 to 12 years.

To be eligible for the study, subjects had to be declared medically fit after a clinical examination, without any history of clinical varicella or zoster infection, free from allergic or adverse reactions to any previous vaccination and should not have received any immunoglobulins or blood products within three months of blood sampling or administration of the study vaccine.

Study design

A pre-vaccination screening was done to ensure the subjects enrolled for the study were seronegative for varicella zoster antibodies (anti-VZV). No controls were used in this study and the duration of the study was approximately two months per subject. On Day 0, a single dose (0.5 ml) of live-attenuated Oka strain varicella vaccine was administered subcutaneously in the upper left arm.

Blood samples were planned to be collected from a subset of 150 subjects (first 50 subjects from each subgroup) for pre- and post-vaccination antibody measurements on the day of vaccination (Day 0) and at Day-42 post-vaccination to analyze the anti-VZV titer.

Vaccines

The vaccine used in this study was developed and manufactured by SmithKline Beecham, currently GlaxoSmithKline (GSK) Biologicals (Rixensart, Belgium). One dose (0.5 ml) of the commercial live attenuated varicella vaccine (*Varilrix*™) contained (after reconstitution with diluent) live-attenuated Oka strain: no less than $10^{3.3}$ pfu/dose. The vaccine was supplied in monodose vials containing a freeze-dried pellet and was reconstituted immediately before use with the diluent supplied with the vaccine. The vaccine was stored at 2-8ºC.

Safety

Diary cards were used by the subjects' parents/guardians to record solicited and unsolicited symptoms. Local symptoms (injection site swelling/soreness/redness) were followed-up over 7 days (Days 0 to 6), fever (axillary temperature ≥37.5ºC) over 3 days (Days 0 to 2) and rash over 43 days post-vaccination. The intensity of local symptoms (swelling and redness) was graded from 0-3 (0 = absent, 1= <5 mm, 2 = 5-20 mm and 3 = >20 mm). The number of papules/vesicles observed were counted and scored as 0, 1-10, 11-30, 31-100, 101-200, and >200. Grade 3 fever was defined as an axillary temperature >39ºC. All other grade 3 symptoms were defined as symptoms that prevented normal daily activities. Any other unsolicited symptoms or serious adverse events (SAE) that might have occurred on the day of vaccination (Day 0) and during the 43-days post-vaccination follow-up period were recorded by the parents/guardians of the subjects and reported to the investigator.

Causality of each adverse event was assessed by the investigator based on the following categories: probable – there was probably a direct cause and effect relationship between the vaccine and the adverse event; suspected – reasonable possibility the adverse event was caused by the vaccine, although a direct cause and effect relationship was not established; unlikely – the study vaccine was not suspected as a cause; not related – the adverse event was definitely not related to the study vaccine.

Serology

The serostatus screening was conducted using an ELISA test kit (Enzygnost® anti-VZV/IgG, Behringwerke Laboratories, Germany) for VZV IgG detection. The antibody titer of the sample was expressed as a reciprocal of the last dilution which tested positive. Serum samples were tested for VZV IgG antibodies, both pre-and post-vaccination at GSK Biologicals' laboratory using an indirect immunofluorescence (IIF) method (the commercial kit: Virgo® VZV IgG Indirect Immunofluorescent antibody test (Pharmacia) was

Table 1
Planned enrollment and attrition.

	Total	Percent	Subgroups 1	2	3
Number of subjects planned	300		100	100	100
Number of subjects enrolled	300	100.0	101	118	80
Study vaccine dose not administered according to protocol	1				1
Essential data missing [a]	1				
Number of subjects included in the ATP analysis of reactogenicity	298	99.3	101	118	79
Initially seropositive or initially unknown antibody status	2				2
Non-compliance with blood sampling schedule (including wrong and unknown date)	12		3	8	1
Obvious incoherence or abnormality or error in data	3			2	1
Subject not planned to be bled for all their blood sampling visits	145		56	61	28
Number of subjects included in the ATP analysis of immunogenicity	136	45.3 [b]	42	47	47

[a] For one subject, age was not known and hence, this subject was not assigned to any of the above subgroups. This subject was eliminated from the ATP reactogenicity analysis.
[b] According to the protocol, immunogenicity analysis was planned only for the first 150 subjects (first 50 subjects from each age group), who came for post-vaccination blood sampling.
Subgroup 1, 10 months to <3 years, Subgroup 2, 3 years to <7 years; Subgroup 3, 7 to 12 years

used with some modifications).

Seroconversion was defined as the appearance of a detectable level of anti-VZV antibodies [≥0.2 A.U. (Arbitrary Units) with the Enzygnost kit or ≥ 4 with the IIF method] in the serum of seronegative subjects (<0.2 A.U.: with the Enzygnost kit or <4 with the IIF method) before vaccination. Geometric mean titers (GMTs) of varicella antibodies and their 95% confidence intervals (CIs) were calculated from pre- and post-vaccination sera. Antibody titers lower than the assay cut-off were given an arbitrary value of one half the cut-off value for GMT calculation.

Statistical methods

The demographic characteristics (age, gender) for each subgroup were tabulated. The mean age (plus range and standard deviation) by gender was calculated per subgroup. The incidence of solicited local symptoms over the 7-day follow-up period, fever over the first 3 days and rash over the 43-day follow-up period after vaccination were calculated. Immunogenicity analysis was planned to be conducted in the first 150 subjects (first 50 subjects from each subgroup). Seroconversion rates and GMTs of VZV antibodies in seroconverters, with 95% CI, were estimated at Day-42 post-vaccination. All analyses were performed using SAS® 6.10 with a type-I error (α) of 5%.

RESULTS

Demographics and attrition

A total of 300 subjects were enrolled: 101 in the 10 months to <3 years old subgroup, 118 in the 3 to <7 years old subgroup and 80 in the 7 to 12 years old subgroup. The age of one subject was unknown, hence, this subject was not assigned to any subgroup. The male:female ratio was 1.04:1.

The evaluable cohort for reactogenicity

Table 2
Demographics of subjects (ATP reactogenicity cohort).

Subgroup[a]	Gender	*N*	Mean age (years)	SD	Min age	Max age
1	Female	47	1.4	0.61	10 months	2 years
	Male	54	1.4	0.48	1 year	2 years
	Total	101	1.4	0.55	10 months	2 years
2	Female	60	4.4	1.18	3 years	6 years
	Male	58	4.4	1.24	3 years	6 years
	Total	118	4.4	1.20	3 years	6 years
3	Female	39	8.5	1.41	7 years	12 years
	Male	40	8.5	1.57	7 years	12 years
	Total	79	8.5	1.48	7 years	12 years
Total	Female	146	4.5	2.95	10 months	12 years
	Male	152	4.4	3.00	1 year	12 years
	Total	298	4.4	2.97	10 months	12 years

[a]1, 10 months to <3 years; 2, 3 to <7 years; 3; 7 to 12 years
N, Total number of subjects; SD, Standard deviation; Min, minimum; Max, maximum

included 298 subjects (Tables 1 and 2); two subjects were eliminated from analysis – vaccine being administered in the wrong site in one subject, while essential data regarding age and reactogenicity was missing in the other.

Immunogenicity analysis was conducted in the first 150 subjects. Subjects who were seropositive prior to vaccination (2 subjects), had incoherence, an abnormality or error in data (3 subjects) and were non-compliant with blood sampling schedule (12 subjects) were eliminated from analysis. A total of 136 subjects were included in the analysis for immunogenicity (Tables 1 and 3).

Reactogenicity

All local injection site symptoms were considered causally related to vaccination. One subject reported a solicited local symptom (grade 1 injection site soreness) which resolved within two days of onset. Fever was more prevalent than rash, occurring in 29 subjects (10%) compared to 3 subjects (1%), respectively (Table 4). A trend towards an increase in the incidence of fever was observed with decreasing age (6.3%, 8.5%, and 14.1%; 7 to 12 years old, 3 to <7 years old, and 10 months to <3 year old subgroups, respectively). In five cases (1.7%) grade 3 (severe) fever were reported (n = 1, 10 months to <3 year old subgroup; n = 4, 3 to <7 year old subgroup), of which 3 were determined by the investigator to have a "probable" or "suspected" relationship to vaccination. These symptoms resolved within a maximum of 5 days from the date of onset.

During the 43-day post-vaccination follow-up period, a total of 23 unsolicited symptoms were reported by 14 subjects. All these were general symptoms, none of which were severe in intensity. Three symptoms (headache and fever in one subject on the day of vaccination and pharyngitis in another subject, two days after vaccination) were determined by the investigator to have

Table 3
Demographics of subjects (ATP immunogenicity cohort).

Subgroup[a]	Gender	*N*	Mean age (years)	SD	Min age	Max age
1	Female	20	1.4	0.60	10 months	2 years
	Male	22	1.3	0.46	1 year	2 years
	Total	42	1.3	0.53	10 months	2 years
2	Female	17	4.4	1.27	3 years	6 years
	Male	30	4.4	1.25	3 years	6 years
	Total	47	4.4	1.24	3 years	6 years
3	Female	20	8.6	1.47	7 years	12 years
	Male	27	8.6	1.55	7 years	12 years
	Total	47	8.6	1.50	7 years	12 years
Total	Female	57	4.8	3.26	10 months	12 years
	Male	79	4.9	3.14	1 year	12 years
	Total	136	4.9	3.18	10 months	12 years

[a]1, 10 months to <3 years; 2, 3 to <7 years; 3 = 7 to 12 years
N, Total number of subjects; SD, Standard deviation; Min, minimum; Max, maximum

a "probable" or "suspected" relationship to vaccination.

No serious adverse events were reported during the study period.

Immunogenicity

Subjects who were seronegative for anti-VZV antibodies prior to vaccination were included in the according-to-protocol (ATP) cohort for immunogenicity (*N* = 136; 42 in the 10 months to <3 year old subgroup, 47 in the 3 to <7 year old subgroup and 47 in the 7 to 12 year old subgroup). Forty-three days post-vaccination, all but one subject seroconverted the having anti-VZV antibodies (Table 5), corresponding to a GMT of 112.6 IIF.

DISCUSSION

The Oka strain varicella vaccine has been extensively assessed in clinical studies worldwide in both healthy and immunocompromised children and has been demonstrated to be well tolerated and immunogenic (Ramkissoon *et al*, 1995; Meurice *et al*, 1996; Tan *et al*, 1996; Varis and Vesikari, 1996). In the current study this vaccine was also found to be well tolerated with no SAEs and immunogenic when given as a primary vaccine to Indonesian subjects aged 10 months to 12 years old.

The very low incidence of unsolicited adverse events reported during the 43-day post-vaccination follow-up period is in line with the results from an earlier study in a similar age group (Diaz-Mitoma *et al*, 2000; Chiu and Lau, 2005). Although symptoms of fever seemed to be more common in the younger age groups (14.1% in the 10 months to <3 year old subgroup and 8.5% in the 3 to <7 year old subgroup), none were regarded as severe. Studies have shown the baseline incidences of fever are higher in children in their early years of life (Offit, 2008).

A single primary vaccination with the Oka strain varicella vaccine proved to be

Table 4
Incidence of solicited general symptoms reported during the 3-day follow-up period for fever and 43-day follow-up period for rash (Total cohort).

Symptom	Subgroup[a]	1 *N* = 99	2 *N* = 117	3 *N* = 80	Total *N* = 297[b]
Fever	All	14.1%	8.5%	6.3%	9.8%
	Grade "3"	1.0%	3.4%	0%	1.7%
	PB/SU	5.1%	6.0%	2.5%	4.7%
	Grade "3" PB/SU	0%	2.6%	0%	2.6%
Rash	All	1.0%	1.7%	0%	1.0%
	Grade "3"	0%	0%	0%	0%
	PB/SU	0%	0%	0%	0%

[a]1, 10 months to <3 years; 2, 3 to <7 years; 3, 7 to 12 years
[b] For subject no. 96 age was unknown, hence, this subject was not assigned to any of the subgroups. This subject did not report any solicited general symptoms.
Grade "3", fever ≥39.0ºC; rash - incapacitating and prevented normal everyday activities
PB/SU, "Probable" or "suspected" relationship to vaccination as determined by the investigator
N, total number of symptom sheets completed

Table 5
Seroconversion (SC) rates and GMTs of anti-varicella antibodies post-vaccination (Day 42) (ATP cohort).

Subgroup[a]	*N*	SC	%	GMT	GMT 95% CI	
					LL	UL
1	42	42	100.0	136.7	102.8	181.9
2	47[b]	45	97.8	122.3	88.6	169.0
3	47	47	100.0	87.2	60.4	125.9
Total	136[b]	134	99.3	112.6	93.2	135.9

[a]1, 10 months to <3 years; 2, 3 to <7 years; 3, 7 to 12 years
[b] For one subject in the 3 to <7 year old subgroup, blood sampling results were not available.
N, Number of subjects tested
SC, Number of subjects who seroconverted for anti-varicella antibodies (antibody titers ≥4 IIF titer)
95% CI, 95% confidence intervals, lower and upper limit

immunogenic in seronegative subjects age from 10 months to 12 years old. The study vaccine was highly immunogenic in all age groups assessed with a high GMT of 112.6 IIF. This is in line with the GMT results of previous studies performed with this varicella vaccine (Barzaga *et al*, 2002). In many Southeast Asian countries, children age 12 months to 12 years old were vaccinated with one dose of varicella vaccine, while two doses were administered to individuals ≥13 years old. It was observed in earlier studies

in children age 12 months to 12 years old, the overall seroconversion rate was ≥97% after one dose of vaccine. About, 86% of the adolescents and adults ≥13 years of age seroconverted after the first vaccine dose, while 99% of them seroconverted after the second vaccine dose which was given a month later (Ampofo, 2002; Barzaga *et al*, 2002; Chiu and Lau, 2005).

The results of this study in healthy seronegative infants and children of different age groups further substantiates the established excellent safety and immunogenicity profile of the Oka strain, refrigerator temperature stable, live-attenuated, varicella virus vaccine. Broader application of this vaccine in countries where vaccines cannot be kept frozen is possible since the reformulated vaccine can be stored at 2-8ºC for a period of two years.

In summary, a routine varicella vaccination program for healthy children is estimated to prevent 94% of all potential cases of varicella, provided the vaccination coverage rate is 97% at school entry (Lieu *et al*, 1994). The data provided in this study are consistent with the safety and immunogenicity profile generated so far for the administration of the Oka strain reformulated varicella vaccine. Given the disease burden observed in children, adolescents and adults, vaccinations seems to be an effective way of controlling the disease.

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