ADVANTAGE OF A TWO-DOSE *VERSUS* ONE-DOSE VARICELLA VACCINE IN HEALTHY NON-IMMUNE TEENAGERS AND YOUNG ADULTS

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Abstract. This study was undertaken to compare the immunogenicity and reactogenicity of two vaccines based on the attenuated Oka-strain of Varicella zoster virus (VZV), in adolescents and young adults. One hundred and eighty-six subjects, aged 13 to 29 years, were randomized to one of two groups to receive a one- or a two-dose VZV vaccine. Pre- and post-vaccination blood samples were assayed for VZV-specific IgG. Solicited local and general symptoms, as well as unsolicited symptoms, were recorded post-vaccination. Seroconversion rates were 94.9% in the one-dose, and 100% in the two-dose, regimen. The two-dose vaccine elicited significantly higher geometric mean antibody titer, 392.5 *vs* 86.8 pfu. Transient local injection site pain was the most frequently-reported symptom per dose in both groups (one dose: 48.9%; two-dose: 32.8%). The two-dose vaccine regimen afforded the advantage of higher antibody titers and potential increased protection from disease, without significantly increased reactogenicity.

INTRODUCTION

Varicella zoster virus (VZV) disease continues to be commonly seen as an irritating but generally mild disease by both the lay population and healthcare professionals. Although typically benign in healthy children, primarily infected adolescents, adults and immunocompromised individuals are at risk of severe complications and occasionally death (Fleisher *et al*, 1981). In temperate climates, most cases occur before the age of 10 and the majority of adults, even those with a negative history for varicella disease, are seropositive for VZV antibody when tested (Rusthoven, 1994). The epidemiology is less well understood in tropical areas, where between 9-40% of adolescents and young adults remain susceptible to infection, a situation that has important health implications owing to the age-related increase in varicella severity (Lee, 1998; Clemens *et al*, 1999; Tregnaghi *et al*, 1999; Lolekha *et al*, 2001).

Vaccines based on the attenuated Oka-strain of VZV have been proven to be safe and efficacious in controlling this disease (Kuter *et al*, 1991; Krause and Klinman, 1995; White, 1997; Burgess *et al*, 1999; Wise *et al*, 2000). The American Academy of Pediatrics and the Advisory Committee for Immunization Practices (ACIP) have included varicella immunization into the routine schedule. Their recommendations for the VZV vaccine in the USA include universal use of one dose in children aged 12 months to 12 years and two doses 4-8 weeks apart in susceptible adolescent and adult populations (ACIP, 1999; Committee on Infectious Diseases, 2000), a recommendation supported by the Centers for Disease Control (Centers for Disease Control and Prevention, 1996; White, 1997). Other countries in which routine childhood vaccination has been adopted include Canada, Japan, Korea, and Uruguay. Although Finland is the only European country to date that has adopted a universal childhood vaccination policy, the European Working Group on Varicella Vaccination (EuroVar) has proposed an immunization strategy consistent with that of the ACIP (Rentier, 2000).

Mathematical modeling, using a range of values for vaccine efficacy at different rates of vaccine coverage, suggest that routine immunization of pre-school children would greatly reduce the number of primary varicella cases (Halloran *et al*, 1994a,b; Halloran, 1996). The World Health Organization (2001) advises that routine childhood immunization be considered in countries where this disease is a relatively important public health and socioeconomic problem, where the vaccine is affordable, and where high -

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85%-90% - and sustained vaccine coverage can be achieved. In addition, immunization of adolescents and adults without a history of varicella, and in particular those at increased risk of contracting and spreading infection, is recommended.

This study was undertaken to assess the immunogenicity and reactogenicity of a one- and a two-dose schedule of two VZV vaccines (both Oka-strain) in susceptible adolescents and young adults.

MATERIALS AND METHODS

One hundred and eighty-six teenagers and young adults (aged 13 to 29) were enrolled into this open prospective study, conducted at Khon Kaen University, Khon Kaen, Thailand. The study was approved by the institutional ethics review board and was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines effective at study initiation. Written informed consent in the local language was obtained from the subjects or parents or guardians (dependent upon the age of the subject) prior to entry into the trial.

Subjects were excluded if they had received previous vaccination against varicella, had a clear history of clinical varicella/zoster infection, prevaccination serum-positive for varicella antibody, as determined by enzyme linked immunosorbent assay (ELISA), or had known exposure to varicella/zoster within four weeks prior to study vaccination. Other exclusion criteria were acute or chronic disease, chronic drug therapy, immunosuppressive therapy or receipt of immunoglobulins or blood products within three months prior to vaccination, history of allergic disease, confirmed or suspected immunodeficient conditions, chronic alcohol consumption and/or intravenous drug abuse. Pregnant or lactating females, or females of childbearing potential who were not using contraceptive precautions, were not included in the study.

Eligible subjects were randomized into one of two groups to receive either Biken Institute (Biken) vaccine which consists of a single dose, according to its prescribing information, or GlaxoSmithKline Biologicals' (GSK) vaccine which is recommended as a two-dose schedule in subjects ≥13 years of age (with a six-week interval between the two vaccinations). Biken vaccine (distributed by Aventis Pasteur) has a potency of not less than 10^3 plaque-forming units (pfu) per dose. Varilrix™ produced by GSK contains $\geq 10^{3.3}$ pfu/dose. Both vaccines were reconstituted before use with the diluent provided by the manufacturer and administered subcutaneously into the non-dominant upper arm.

Pre- and post-vaccination blood samples were assayed for varicella zoster (VZV)-specific IgG, using a commercial indirect immunofluorescence (IIF) technique (Virgo™ by Pharmacia). Samples that showed no fluorescence or barely visible fluorescence at the 1:4 starting dilution were considered seronegative. Seroconversion was defined as the appearance of antibodies in the serum of subjects who were initially seronegative (*ie* IIF titer ≥1:4 in the serum of a subject who was previously seronegative).

Local injection site symptoms (pain, redness, and swelling) were solicited on the day of vaccination and for three subsequent days. All vaccinees were followed for 42 days after vaccination for the occurrence of general symptoms (fever defined as axillary temperature ≥37.5°C and rash/exanthem). Subjects were asked to record temperature daily and any other findings on diary cards, and to contact the investigator immediately if they developed any rash at the injection site or generalized rash, if they were exposed to anyone with varicella/zoster, or if they felt any symptom they thought was serious or required medical attention. Any post-vaccination rash was evaluated and its relationship to vaccination determined by the investigator.

The sample size was determined based upon the expected ability to accrue seronegative adults. Fisher's exact test was used to compare seroconversion rates and the incidence of symptoms. Wilcoxon's test was used to compare geometric mean titers (GMT) of anti-VZV, which were calculated using log transformation of positive titers and taking the antilog of the mean of the transformed titers. Alpha was 0.05.

RESULTS

The mean age of the study cohort was 16.9 years, with a standard deviation of ± 3.79 years, and a male:female ratio of 1:3.3. The two groups did not differ with respect to age or gender distribution (Table 1). Of the 186 subjects enrolled and randomized, 32 were not eligible for inclusion in the analysis of immunogenicity. Seventeen subjects (11 in the two-dose regimen group, and 6 in

the one-dose regimen group) were eliminated from analysis owing to anti-VZV seropositivity according to the IIF assay performed at first visit. Two subjects in the two-dose regimen group were eliminated due to unknown serostatus. Twelve subjects (7 in the two-dose regimen group, and 5 in the one-dose regimen group) failed to comply with the blood sampling schedule, and one subject in the two-dose regimen group had received a concomitant tetanus vaccine.

Of the remaining 154 seronegative subjects, 73 received the two-dose GSK regimen and 81 received the one-dose Biken regimen. The one-dose Biken vaccine elicited 94.9% seroconversion, while the two-dose GSK vaccine resulted in 100% seroconversion in these naïve subjects (Table 2). The level of significance was 0.1212, as determined by Fisher's exact test.

The GSK vaccine yielded significantly higher GMT of post-vaccination varicella antibodies; 392.5 *vs* 86.8 in the Biken group. Wilcoxon's test showed a statistically significant difference (p=0.0001).

Symptoms were reported following 64.1% of doses in the one-dose regimen and 56.3% of doses in the two-dose regimen (Table 3). Transient local injection site pain, the most common side-effect in both groups, was reported following 48.9% of doses in the one-dose regimen, and 32.8% of doses in the two-dose regimen. Most symptoms were described as easily tolerated by both groups and no serious adverse event occurred in either group. Clinically significant or Grade 3 pain was reported following 4 of the total 183 vaccinations in the two-dose regimen and 3 of the total 92 vaccinations in the one-dose regimen.

Over the 42 days of follow-up after each dose, fever occurred following 20.9% of doses in the one-dose regimen and 24.9% of doses in the two-dose regimen (p=0.5452).

Four episodes of rash were reported (one in the one-dose regimen group and three in the two-dose regimen group), none of which was described as varicella-like by the investigator. However, all were attributed a suspected/probable link with vaccination.

Table 1
Subject distribution and demography of subjects included in the analysis of immunogenicity.

	2-dose vaccine N = 73	1-dose vaccine N = 81
Mean age and range (years)	16.6 ± 3.72	16.6 ± 3.57
Gender ratio (M/F)	16/57	21/60

N: Number of subjects

DISCUSSION

The one-dose Biken vaccine and the two-dose GSK vaccine elicited seroconversion rates of 94.9% and 100%, respectively. The general presumption has been that seroconversion after administration of a live attenuated viral vaccine correlates with protection from natural disease. In reality, data indicate that seroconversion does not always render protection from disease. Rather, the more robust the antibody response after varicella vaccination, as was elicited by the two-dose vaccine in this study, the less likely the individual is to have breakthrough disease in the following years (White *et al*, 1992).

In this study, the level of antibody response to the two-dose vaccine was clinically and statistically significantly higher than the single dose vaccine.

Humoral responses to the VZV vaccine have been measured by various assays, including immune adherence hemagglutination assay, fluorescent antibody to membrane antigen assay, en-

Table 2
Seroconversion rates and geometric mean titers of VZV antibody after varicella vaccination.

Vaccination regimen	N	Seroconversion		GMT	
		%	95% CI	Value	95% CI
1-dose vaccine	81	94.9	87.5;98.6	86.8	63.6;118.6
2-dose vaccine	73	100.0	95.1;100.0	392.5	318.7;483.3

N: Number of subjects tested; CI: Confidence interval

Table 3
Incidence of symptoms per number of doses of vaccine.

Symptoms	GSK (N = 183) n (%)	Biken (N = 92) n (%)	Fisher's exact test p-value
Any symptom (solicited/unsolicited)	103 (56.3)	59 (64.1)	0.2431
Local injection site symptoms solicited until Day 3 after vaccination			
Pain			
Total	60 (32.8)	45 (48.9)	0.0123[a]
Prevented normal daily activity[b]	4 (2.2)	3 (3.3)	0.6899
Redness			
Total	26 (14.2)	17 (18.5)	0.3816
>20 mm diameter	1 (0.5)	0 (0.0)	>0.9999
Swelling			
Total	24 (13.1)	17 (18.5)	0.2819
>20 mm diameter	0 (0.0)	0 (0.0)	1.0000
General symptoms solicited until Day 42 after vaccination			
Fever			
≥37.5°C	45 (24.9)	19 (20.9)	0.5452
>39°C	2 (1.1)	1 (1.1)	>0.9999
Rash			
Total	3 (1.7)	1 (1.1)	>0.9999
Varicella like[c]	0 (0.0)	0 (0.0)	1.0000

N: number of documented doses; n (%): number/percentage of doses followed by the specific symptom
[a]Statistically significant; [b]clinically significant or Grade 3; [c]papulovesicular or vesicular rash

zyme-linked immunosorbent assay (ELISA) and glycoprotein-based ELISA (gpELISA).

Antibody titer, as measured by gpELISA in the six weeks following vaccination, has been used as a surrogate marker for protection against natural disease (White *et al*, 1992; Bernstein *et al*, 1993; Krause and Klinman, 1995; Gershon, 1998; Shaw, 2000). Low-level antibody response (titer <5 U) has been associated with breakthrough infection, although with generally milder disease than after natural infection (Plotkin, 1996; White, 1997).

A report of a 10-year survey of the Biken vaccine, as used in Japan, confirmed that a correlation also exists between the degree of protection and the height of antibody response, as measured by immune adherence hemagglutination assay, *ie*, the likelihood of developing varicella post-vaccination is in inverse proportion to the concentration of antibody. In fact, the reporters concluded that the administration of a booster dose, *ie*, a second dose of vaccine, would likely decrease the incidence of breakthrough varicella (Ozaki *et al*, 2000).

Although breakthrough cases of varicella in vaccinees are generally mild, these factors need to be considered when implementing a vaccination program. Because latent infection is likely to be related to the skin lesions seen in varicella, reducing breakthrough varicella seen in those vaccinated might also play a role in decreasing the incidence of herpes zoster (Lim *et al*, 1998).

The immunofluorescence assay used in this trial is specific for IgG class antibody, which has some VZV-neutralizing activity and, when developed in response to wild virus infection, persists indefinitely. Long-term immunity is thought to be conferred by the persisting IgG antibodies, as well as cell-mediated immunity (White, 1997).

Since it has been demonstrated that measurement of humoral VZV antibody concentration provides the best determinant of response to vaccine and defense from disease, the two-dose vaccine performed more effectively than the one-dose vaccine.

Aside from the obvious fact that one group received one more injection than the other, both vaccines gave rise to local and general side-effects of the same type and intensity. The incidence and type of symptoms in both groups were consistent with these previously reported for inactivated VZV vaccines (Clements, 2000; Diaz-

Mitoma *et al*, 2000).

Results of this study indicate that the two-dose vaccine utilized in this study afforded the advantage of higher initial antibody response and thereby the potential for increased protection from disease without significantly increased reactogenicity.

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REFERENCES

ACIP, Prevention of varicella updated recommendations of the Advisory Committee on Immunization Practices. *MMWR* 1999; 48 (RR06): 1-5.

Bernstein HH, Rothstein EP, Watson BM, *et al*. Clinical survey of natural varicella compared with breakthrough varicella after immunization with live attenuated Oka/Merck varicella vaccine. *Pediatrics* 1993; 92: 833-7.

Burgess MA, Cossart YE, Wilkins TD, Botham S, Fearns G, Chitour K. Varicella vaccination of health-care workers. *Vaccine* 1999; 17: 765-9.

Centers for Disease Control and Prevention. Prevention of varicella. *MMWR* 1996; 45 (RR-11): 1-25.

Clemens SA, Azvedo T, Fonseca JC, Cavalcante Silva A, de Silveira TR, Clemens R. Seroepidemiology of varicella in Brazil – results of a prospective, cross-sectional study. *J Pediatr* (Rio J) 1999; 75: 433-41.

Clements DA. Varicella vaccination in children. *Biodrugs* 2000; 14: 49-60.

Committee on Infectious Diseases, American Academy of Pediatrics. Varicella vaccine update. *Pediatrics* 2000; 105: 136-41.

Diaz-Mitoma F, Halperin SA, Scheifele D. Reactogenicity to a live attenuated varicella vaccine in Canadian children. *Can J Infect Dis* 2000; 11: 97-102.

Fleisher G, Henry W, McSorley M, Arbeter A, Plotkin S. Life-threatening complications of varicella. *Am J Dis Child* 1981; 135: 896-9.

Gershon AA. The impact of varicella vaccine: present and future. *Herpes* 1998; 5: 72-4.

Halloran ME, Cochi SL, Lieu TA, Wharton M, Fehrs L. Theoretical epidemiologic and mobidity effects of routine varicella immunization of preschool children in the United States. *Am J Epidemiol* 1994a; 140: 81-104.

Halloran ME, Watelet L, Struchiner CJ. Epidemiologic effects of vaccines with complex direct effects in an age-structured population. *Math Biosci* 1994b; 121: 193-225.

Halloran ME. Epidemiologic effects of varicella vaccination. *Infect Dis Clin North Am* 1996; 10: 631-55.

Krause PR, Klinman DM. Efficacy, immunogenicity and use of live attenuated chickenpox vaccine. *J Pediatr* 1995; 127: 518-25.

Kuter BJ, Weibel RE, Guess HA, *et al*. Oka/Merck varicella vaccine in healthy children: final report of a 2-year efficacy study and 7-year follow-up studies. *Vaccine* 1991; 9: 643-7.

Lee BW. Review of varicella zoster seroepidemiology in India and Southeast Asia. *Trop Med Int Health* 1998; 3: 886-90.

Lim YJ, Chew FT, Tan AYS, Lee BW. Risk factors for breakthrough varicella in healthy children. *Arch Dis Child* 1998; 79: 478-80.

Lolekha S, Tanthiphabha W, Sornchai P, *et al*. Effect of climatic factors and population density on varicella zoster virus epidemiology within a tropical country. *Am J Trop Med Hyg* 2001; 64: 131-6.

Ozaki T, Nishimura N, Kajita Y. Experience with live attenuated varicella vaccine (Oka strain) in healthy Japanese subjects; 10-year survey at pediatric clinic. *Vaccine* 2000; 18: 2375-80.

Plotkin SA. Varicella vaccine. *Pediatrics* 1996; 97: 251-3.

Rentier B. Varicella vaccination: building on US experience to promote European recommendations [Abstract]. Vaccination in Progress, Progress in Vaccination, Session 1. October 2000.

Rusthoven JJ. The risk of varicella-zoster infections in different patient populations: a critical review. *Transfu Med Rev* 1994; 8: 96-116.

Shaw AR. What defines protection from varicella? [Abstract]. Buenos Aires, Argentina: 9th International Congress on Infectious Diseases. April 2000.

Tregnaghi M, Lagos R, Urdaneta E, *et al*. Epidemiological serosurvey of varicella in Latin America [Abstract]. 2nd World Congress of Pediatric Infectious Diseases, November 1999: 34.

White CJ, Kuter BJ, Ngai A, *et al*. Modified cases of chicken pox after varicella vaccination: correlation of protection with antibody response. *Pediatr Infect Dis J* 1992; 11: 19-23.

White CJ. Varicella zoster virus vaccine. *Clin Infect Dis* 1997; 24: 753-63.

WHO. The WHO Position Paper on Varicella Vaccines, 2001. [last visited 12-06-03]. Available from: URL: http://www.who.int/vaccines-diseases/diseases/PP_Varicella.htm

Wise RP, Salive ME, Braun MM, *et al*. Postlicensure safety surveillance for varicella vaccine. *J Am Med Assoc* 2000; 284: 1271-9.