A Comparative Assessment of the Immunogenicity and Reactogenicity of Two Oka Strain Based Live Attenuated Varicella Vaccines

Hyunsoo Kim

The Graduate School
Yonsei University
Department of Public Health

A Comparative Assessment of the Immunogenicity and Reactogenicity of Two Oka Strain Based Live Attenuated Varicella Vaccines

A Master's Thesis
Submitted to the Department of Public Health
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Master of Public Health

Hyunsoo Kim

December 2001

This certifies that the master's thesis of Hyunsoo Kim is approved.

Thesis Supervisor: Hee-Choul Ohrr

Young-Mo Sohn

Jae-Hoon Roh

The Graduate School

Yonsei University

December 2001

Dedication

As I have almost ten years experience in the pharmaceutical field, I have enjoyed specially work of vaccine field very much. I think this thesis is a result from my private interest in this field. I have enjoyed my study related with my work recent two years.

I especially appreciate Dr. Hee-Choul Ohrr who gave me the wonderful opportunity to study this field. I also thank to Dr. Young-Mo Sohn and Dr. Jae-Hoon Roh who gave me a lot of guidance when I wrote this thesis.

I also thank to my colleagues working with me in my company and my family, specially my wife Jin-Seon.

December, 2001

Hyunsoo Kim

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT IN ENGLISH

A Comparative Assessment of the Immunogenicity and Reactogenicity of Two Oka Strain Based Live Attenuated Varicella Vaccines

Three manufacturer's Oka strain derived live attenuated varicella vaccines ("Oka vaccine") have been licensed in China and implemented on regional basis for the children under 10 years of age. A randomized, double-blinded, comparative trial was performed from March to August in 1999 to investigate the immunogenicity and reactogenicity of two Oka vaccines in 174 children resident in Guangxi, China. Two anti-epidemic public health stations recruited healthy children at ages of 1 to 6 years, who were then randomly allocated into one of two comparative groups. One group received one dose of Changchun vaccine (lot no. 980544) containing a mean virus titer of 4.1 log PFU per dose, whereas the other group were immunized with the vaccine supplied by an international vaccine manufacturer (lot no. VA201A44A) with a mean virus titer of 4.9 log PFU per dose. Clinical signs and symptoms were observed on every 5 days during 42 days following vaccination.

By day 42 post-vaccination 100% of the initially seronegative subjects given with Changchun vaccine (group 1), and 94.9% with reference vaccine (group 2) had shown antibody response. The seroconversion rate of the two

groups was not significantly different to each other (Fisher's exact test: $p > 0.05$). The post-vaccination GMT of the group given with Changchun vaccine was 47.4 whereas 11.4 in the reference vaccine.

No vesicular rash or any other serious adverse events were reported among the recipients of either vaccine. One mild pain and one pruritus were reported from group 1, whereas a mild pain accompanied by pruritus and one redness case was reported from group 2. All symptoms above disappeared within three days. There was no significant difference in the frequency of signs and symptoms in the two comparative groups (Chi-square test: $p > 0.1$).

---

varicella vaccine, immunogenicity, seroconversion rate, reactogenicity, side effect

1. BACKGROUND

Primary infection by varicella-zoster virus causes varicella (chicken pox), which is usually a benign disease of childhood. However, varicella is highly contagious and infection rate of household contact is about 90%. Primary prevention by vaccination has initially done for the immunocompromised patients in whom the infection may cause severe manifestations and complications eventually leading to death [1, 2]. The clinical studies have shown that vaccination program against varicella of all healthy children at early age has also several advantages; it can prevent varicella complications, a reduction in the spread of varicella particularly in institutions as well as cost-benefits. Also, it has been postulated that there would be a reduction in the occurrence and severity of zoster in later life [1-4].

In the early 1970's, a live-attenuated varicella vaccine designated the Oka strain was developed by Takahashi and colleagues in Japan [5]. The vaccine is a cell-free propagation of OKA strain VZV. The original virus was obtained from an otherwise healthy Japanese boy with natural varicella and was propagated in human embryonic lung fibroblasts, guinea pig embryonic cells, and finally in two different cell strains of human diploid cell cultures (WI-38, MRC-5). Studies have shown that the vaccine is safe, immunogenic, and highly protective against severe varicella infection in healthy children and adults [6, 7]. The varicella vaccine is licensed for routine use in Japan and Korea, where over 2 million doses have been given. It is also licensed in

several European nations for use in immunocompromised children and in 1995 in the United States. In China, the Oka strain based live-attenuated varicella vaccine ("Oka vaccine") was licensed by the State and Drug Administration for use in individuals 12 months of age or older who have not had varicella since late 1990s. Three manufacturers including two Chinese manufacturers and an international supplier have implemented the Oka vaccine, of which the annual number of doses distributed has been dramatically increasing in recent years.

Changchun Institute of Biological Products, one of major Chinese vaccine manufacturer, has developed a similar vaccine based on the Oka strain under the collaboration with the research team of BR BIOTECH (Seoul, Korea). Pre-licensure clinical studies suggest the Changchun vaccine is tolerable and immunogenic in healthy children [8]. The recommended dose of the vaccine, 0.5 ml, contains not < 1400 plaque-forming units of VZV, as well as trace amounts of neomycin. This lyophilized preparation of Changchun vaccine can be stored in 2 to 8 °C whereas the imported Oka vaccine manufactured by an international vaccine supplier ("reference vaccine") should be kept frozen with a temperature of –15°C or colder. A storage life of up to 18 months is guaranteed for both vaccines. Several stability tests have reported that the Changchun vaccine is very stable to heat exposure under the different temperature conditions and storage periods, even after exposure to 37°C for 28 days [9]. When the vaccine sample is kept at

8°C in 30-month period, one dose still contains higher than 1,400 PFU, the minimum virus titer [9]. There might be some advantages in being able to keep the vaccine in the refrigerator rather than the freezer in the countries where hospital logistics, transportation or shipment infrastructure is poor. The vaccine may produce consistently high immune response in the recipients of vaccines suggesting its potential of being utilized in tropical countries in Asia. An initiative was made to re-confirm the efficacy and safety of this potentially interesting Oka strain based live attenuated varicella vaccine. Since the Oka strain based varicella vaccines are shown to be effective and licensed in many countries, a placebo-controlled efficacy trial may be unethical. A randomized, double-blinded, controlled trial is performed to evaluate the immunogenicity and reactogenicity of Changchun vaccine in healthy children 1 to 6 years of age compared with a reference vaccine known to be highly efficacious.

2. MATERIALS AND METHODS

2.1 The Vaccine

Two Oka strain based, live attenuated varicella vaccines were used in this study. A random lot of 980544 manufactured by Changchun Institute of Biological Products, China ("Changchun vaccine") and the reference vaccine supplied by an international vaccine manufacturer ("reference vaccine", lot no. VA 201A44A) were used.

Table 1. Each of Two Groups Received a Dose of the Assigned Vaccine

Group	Manufacturer	Lot No.	Mean Virus titer/dose
1	Changchun	980544	4.1 log pfu
2	An international supplier	VA 201A44A	4.9 log pfu

The vaccines were supplied in single dose vials, each being reconstituted with an individual vial of water for injection. Half ml dose was administered subcutaneously in the left upper arm of study subjects.

2.2 Study Population

Volunteered healthy Chinese children, 1 to 6 years old, resident in Guanyang county of Guangxi province were enrolled for this study.

Inclusion criteria were the followings:

One to six years of age at the time of enrollment

Free from obvious health problem as established by clinical examination

Exclusion criteria were the followings:

History of clinical varicella infection

Significant exposure to varicella or zoster within the preceding 4 weeks

History or evidence of allergy to neomycin and kanamycin

History of allergic or other adverse reaction to any previous vaccination

Any acute febrile illness at the time of vaccination

Any chronic drug therapy to be continued during the study period

Any confirmed or suspected immunosuppression

History of vaccination with other inactivated vaccine within the preceding 4 days

History of vaccination with other live viral vaccine within the preceding 4 days

2.3 Study Design

A randomized, double blind, comparative controlled trial was designed, executed and analyzed. Both Changchun vaccine and reference vaccine were packaged in single dose vial and each of them was labeled as A or B.

The coding had been kept and used for the entire trial period until the time of data analysis. One hundred seventy four children were enrolled at two anti-epidemic stations: they were allocated a study number in the order in which they were enrolled. A randomization list was created using Random Permuted Block method with the table of random numbers for the first 140 subjects in the sequence of patient assignment. The subjects recruited after were allocated to the group of Changchun vaccine due to the limited number of reference vaccine doses. An envelope containing the randomization list was kept by the principal investigator and delivered to both anti-epidemic stations one day ahead the vaccination date and opened by the study clinician.

When a study subject was enrolled, a standard Chinese physical examination was made with body temperature recorded. A pre-vaccination serum sample was collected and one dose of the varicella vaccine was administered subcutaneously in the upper left arm. During the 42 day observation, parents or guardian were asked to observe their children for any clinical signs or symptoms. If rash/eruption or any other serious adverse event occurred, the parents were asked to report to the investigators immediately. At every 5 days, several field workers contacted each household by telephone, or asked them to visit, to evaluate the presence of clinical signs and symptoms. On day 42 a physical examination took place, a post-vaccination serum was collected. All clinical signs or symptoms reported were recorded on a pre-structured form. Any medications

received by the children during the study period were also recorded.

2.4 Ethical Consideration

This study was conducted in accordance with the principles laid down by the World Health Assembly of 1975 on Ethics in Human Experimentation and the Helsinki Declaration The Ethics Review Committee of the Chinese State Drug Administration approved the study protocol [10] [11]. Written informed consent was obtained from the parents or guardian of study subjects prior to enrollment.

2.5 Laboratory Analysis

Separated pre- and post-vaccination serum samples were stored at –20°C until they were titrated in National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). Specific varicella antibodies were measured by FAMA method [12]. Doubling dilution of serum in PBS, beginning at 1:2, are made in a 96-well round bottom micro titer plate. Cells infected with VZV are harvested when they exhibit 80% CPE. Medium is poured off the cultures and the cells are scrapped off the glass into any remaining medium and centrifuged at 700 X g for 2 min. The cells are taken up in PBS (pH 7.2) and dispersed in 0.020 ml aliquots into micro titer plates containing 0.020 ml dilution of serum. Cells are then incubated for 30 min at

37°C in a moist chamber. PBS is added to fill the wells and the plate is centrifuged at 1 000 X g at 4 °C for 10 min, after which the PBS is removed by rapidly flipping the plate upside down. The washing steps were repeated two more times. A working dilution of flurescein-labeled antihuman immunoglobulin is added to each well, and incubation for 30 min at 37°C is again carried out.

After two washes, a drop of buffered glycerol is added to each well, and the cells are removed with an aspirator and placed on microscope slides. The cells are covered with a glass cover slip sealed with nail polish. The slides are then examined by fluorescence microscopy. The titer was expressed as the reciprocal of the last dilution that showed positive. Seroconversion is defined as the appearance of antibodies in the serum of subjects who were seronegative before vaccination (titer < 4 before vaccination to ≥ 4 in post-vaccination serum sample).

2.6 Statistical Methods

2.6.1 Sample Size Plan

The ability to detect a smallest detectable proportion difference of antibody response rate after vaccination depends on the following criteria:

The accepted level of probability of rejecting the null hypothesis (no difference in antibody response rate after vaccination between two groups) (α)

The accepted level of probability of failing to reject the null hypothesis, when in reality, there is a difference (β)

The smallest detectable proportion difference (P1 – P0: 0.05 or 0.10)

The seroconversion rate in the reference vaccine group estimated from the previous studies (P0: 0.95)

5% is assumed as significant smallest difference of immune response after vaccination so that the seroconversion rate of 90% is deemed as inferior to that of reference vaccine. Therefore at least 371 subjects in each group should be enrolled so that the study can achieve 80% power to detect the proportion difference of 0.05 versus an equal proportion using a Fisher's exact test with 0.05% significance level. It was not feasible however to recruit such a large number of study subjects due to the limitation of logistics, time and cost. Assuming 10% is a significant small difference of immune response after vaccination and accordingly 85% of seroconversion rate is deemed as inferior to that of reference vaccine, only 126 subjects in each group are required.

2.6.2 Demographics

The age and sex of study subjects of both groups were summarized; the mean ages of each group were compared using two sample t-test.

2.6.3 Immunogenicity

The percentage of seroconversion and the geometric mean titer (GMT) of specific varicella antibodies in the initially seronegative subjects were calculated. Since the sample size was small, the Fisher's exact test was used to compare two binominal proportions of the seroconversion rates. GMTs of specific varicella antibodies were calculated at the second time point of study (day 42) in post-sera positive subjects using the log transformation of seropostive titres and taking the anti-log of the mean of these transformed values.

2.6.4 Safety and Reactogenicity

The occurrence of solicited signs and symptoms were counted and described as percentage of subjects among the total study subjects in each group. The frequency of signs and symptoms of the two groups was compared using chi-square test.

3. RESULTS

One hundred and seventy four children were enrolled into the study and all received a single dose of vaccine (Figure 1). All of 174 children were included in reactogenicity analysis. Only 128 were included for immunogenicity analysis; 32 children were not included in the analysis either because parents did not agree with the second bleeding of their children or serological data were missed. Further 14 children were not analyzed for immunogenicity as they had seropositive sera at pre-vaccination. The mean age of two comparison groups included in the analysis of immunogenicity was not significantly different from one group to another (two sample t-test, $p > 0.05$). It was shown that two groups were comparable in terms of age and sex from the demographic information.

Figure 1. Number of Enrolled Subjects

	Group 1 (Changchun vaccine)	Group 2 (reference vaccine)	Total
Total number Randomized	115	81	196
↓			
Enrolled in the Study	104	70	174
↓			
Exclusion due to: Missing CRF			
↓			
Analyzed for reactogenicity	104	70	174
↓			
Exclusion due to:			
1) No second blood sample collection	24	8	32
2) Serum positive at pre-vaccination	11	3	14
Included for immunogenicity	69	59	128

3.1 Immunogenicity

3.1.1 Subjects Included in the Analysis

A total of 128 subjects were included in the analysis as they were seronegative before vaccination. Table 2 shows the seroconversion rates and geometric mean titres (GMT) of specific varicella antibodies at day 42 post-vaccination for the 128 initially seronegative subjects. All but three subjects of the 128 initially seronegative children had detectable antibody titers post vaccination. The seroconversion rate of group 1 was 100%, while the group 2 was 94.9%. The seroconversion rates were not significantly different from one group to another (Fisher's exact test: $p > 0.05$). The post-vaccination GMTs were 47.4 and 11.4 for Changchun vaccine and reference vaccine, respectively.

Table 2. Seroconversion Rate (%) and Geometric Mean Titers (GMT) of Antibodies against Varicella for Initially Seronegative Subjects in the Analysis of Immunogenicity

Group	Timing	N	S+	%	GMT	95% CI (Lower)	95% CI (Upper)
1 (Changchun vaccine)	d-42	69	69	100	47.4	11.08	197.4
2 (reference vaccine)	d-42	59	56	94.9	11.4	1.88	66.83

3.1.2 Subjects Excluded from the Analysis

A total of 46 subjects were excluded from the immunogenicity analysis according to the protocol.

3.2 Safety and Reactogenicity

All 174 children were included in the reactogenicity analysis. All symptom sheets were returned after the observation period. During the 42-day follow-up period, parents or guardian of the vaccinee were interviewed at every five days after vaccination for any solicited or unsolicited signs and symptoms. All occurrences of signs or symptoms were recorded (Table 3, 4). There was no significant difference in the frequency of signs and symptoms between two groups (Chi-square test: $p > 0.1$). No serious adverse event was reported among the vaccinees.

Table 3. Subjects with and without Symptoms after Vaccination

Group	N	With symptoms (n)	%	Without symptoms (n)	%
1 (Changchun vaccine)	104	8	7.7	96	92.3
2 (reference vaccine)	70	4	5.7	66	94.3

3.2.1 Solicited Signs and Symptoms

Mild pain was reported in two subjects and pruritus in one subject of test group. Pain accompanied by pruritus was reported in one subject and redness in another child among the study subjects of reference vaccine group. The above symptoms were disappeared within three days. One subject of test group had fever during three days but diagnosed as having tonsillitis.

Under the treatment with antibiotics, his temperature was recovered to normal range, and others symptoms disappeared. A total of 4 children of group 1 had developed fever, but were diagnosed as acute respiratory infection. Two children of reference vaccine group have reported fever but were diagnosed as acute respiratory infection. No person in both groups showed any vesicular rash during observation period.

Table 4. Incidence of Solicited Signs and Symptoms Reported

Symptoms	Group 1 (Changchun vaccine)		Group 2 (reference vaccine)	
	N	%	N	%
Pain	2	1.92	1*	1.4*
Pruritus	1	0.96	1*	1.4*
Redness	0	0	1	1.4
Skin eruption	0	0	0	0
Induration	0	0	0	0
Swelling	0	0	0	0
Fever	5	4.8	2	2.8
Restlessness	0	0	0	0
Headache	0	0	0	0
Rash	0	0	0	0
Vomiting	0	0	0	0

1. The same child showed both symptoms of pain and pruritus (*)
2. All p values are not less than 0.05; no significant difference was found in any solicited signs and symptoms

3. 2. 2 Unsolicited Signs and Symptoms

There were no reported cases of unsolicited signs and symptoms during the 42-day follow-up period after vaccination.

3. 2. 3 Serious Adverse Events

No serious event was reported.

4. DISCUSSION

During March to August in 1999, a total of 174 Chinese children resident in Guangxi province, 1 to 6 years old, participated in this study. Two Oka strain based live attenuated varicella vaccines were used for comparative assessment of safety and immunogenicity; one is the varicella vaccine supplied by the Changchun Institute of Biological Products ("Changchun vaccine") and the other reference vaccine supplied by an international vaccine manufacturer ("reference vaccine").

We found that the Changchun vaccine is equivalent in immunogenicity with that of the reference vaccine after vaccination (100% versus 94.9%, p value > 0.05) and GMT (47.4 versus 11.4) though the power of this study in detecting the small difference of immune response percentage was small (44%). Since the alternative hypothesis was based on the inferior immunogenicity of Changchun vaccine to reference vaccine, which is the opposite direction of this study result, this may not preclude the value of our study.

Previous studies for similar varicella vaccine based on the same Oka strain have reported that vaccination against varicella might induce skin reactions in some children such as rash or vesicles. However, there was no child reporting of rash or vesicles in this study. Pain, pruritus, and redness were reported as the most common local reactions after vaccination in previous clinical studies for other live attenuated varicella vaccines. There

was no significant difference in the frequency of the above solicited signs and symptoms between two groups. Both vaccines were very well tolerated and safe in healthy children. This study is assuring the previous findings that oka-strain based varicella vaccines are well tolerated and the Changchun vaccine is equivalently immunogenic compared to the reference vaccine that is known to be efficacious.

REFERENCES

1. Flesher, G., W. Henry, and M. M, *Life threatening complications of varicella.* Am J Dis Child, 1981. **135**: p. 896-899.
2. Preblud, S.R., W.A. Orenstein, and K.J. Bart, *Varicella: Clinical manifestations, epidemiology and health impact in children.* Pediatri Infect Dis, 1984. **3**(6): p. 505-509.
3. Andre, F.E., *Worldwide experience with Oka strain live varicella vaccine.* Postgrad Med J, 1985. **61**(Suppl 4): p. 113-120.
4. Preblud, S.R., W.A. Orenstein, and J.P. Koplan, *A cost-benefit analysis of a childhood varicella vaccination programme.* Postgrad Med J, 1985. **17-22**(Suppl. 4): p. 17-22.
5. Takahashi, M., T. Otsuka, and Y. Okuno, *Live vaccine used to prevent the spread of varicella in children in hospital.* Lancet, 1974. **2**: p. 1288-1290.
6. Weibel, R.E., B.J. Neff, and B.J. Kuter, *Live attenuated varicella virus vaccine: efficacy trial in healthy children.* N Engl J Med, 1984. **310**: p. 1409-1415.
7. Gershon, A.A., S.P. Steinberg, and P. La Russa, *Immunisation of healthy adults with live attenuated varicella vaccine.* Journal of Infectious Disease, 1988. **158**: p. 132-37.
8. Nan, Y.F., *An open study for tolerability and immunogenicity of an Oka-strain based live-attenuated varicella vaccine in*

Chinese children. Unpublished report, 1992.

9. Nan, Y.F., *An updated stability report of an Oka-strain based live-attenuated varicella vaccine.* Unpublished report, 1999.

10. World Medical Association, *Declaration of Helsinki.* Ethics of research with human subjects, ed. J.P. Kahn. 1998, Frederick: University Publishing Group. 14-18.

11. Levine, R., *Ethics and regulation of clinical research.* 1986, New Haven: Yale University Press.

12. Anne, A.G., *Varicella-zoster virus.* Manual of clinical microbiology, ed. A.P. Michael. 1995, Washington: American Society for Microbiology.

국문 요약

오카 주(Oka Strain) 유래 두 가지 약독 생 수두 백신의 면역원성 및 부작용에 대한 비교 평가

연세대학교 대학원

보건학과

김 현 수

중국에서는 세 곳의 제조사에 의해 생산된 오카 주(Oka Strain) 유래의 약독 생 수두 백신이 허가를 받아 10세 이하의 아동을 대상으로 지역적으로 접종되고 있다. 1999년 3월부터 8월까지 중국 광서 장족 자치구에 거주하는 174명의 아동을 대상으로 두 가지 오카 주 백신에 대한 면역원성과 부작용을 조사하기 위하여 무작위, 이중맹검법의 비교 연구를 시행하였다. 두 개의 보건소에서 1-6세의 건강한 아동을 모집하여 두 개의 비교집단 중 하나에 무작위로 배정하였다. 한 집단은 도스 당 4.1 log PFU의 평균 백신 역가를 함유한 장춘 백신 (lot no. 980544) 한 도스씩을 접종하였고, 다른 집단은 도스 당 4.9 log PFU의 평균 백신 역가를 함유한 국제적 백신 제조사의 백신 (lot no. VA201A44A) 한 도스씩을 접종하였다. 백신 접종 후 5일 마다 42일 동안 임상적 징후와 소견을 관찰하였다.

백신 접종 후 42일 째 장춘 백신 (제1군)을 접종한 최초 혈청

검사시 음성이었던 접종 대상자 100%, 참조 백신 (제2군)을 접종한 94.9%가 항체 양성 반응을 보였다. 두 집단 간의 항체 양성 전환율은 유의성 있게 다르지 않았다. (Fisher's exact test: $p>0.05$). 백신 접종 후 장춘 백신 접종군의 GMT가 47.4인 반면에 참조군의 GMT는 11.4로 나타났다.

두 백신 접종군 모두에서 백신 접종 후 수포성 발진 등 어떠한 심각한 부작용도 나타나지 않았다. 제1군에서 경미한 통증 1예와 소양증 1예가 나타났으며, 제2군에서는 소양증을 동반한 경미한 통증 1예와 홍반 1예가 접종 후 나타났다. 이러한 경미한 증상은 모든 경우에서 3일 이내에 없어졌다. 두 비교 집단 간에 부작용 발생 빈도에 있어서 유의성 있는 차이가 보이지 않았다 (Chi-square test: $p>0.1$).

수두 백신, 면역원성, 항체 양성 전환율, 부작용