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Immunogenicity and safety of a live attenuated varicella vaccine in healthy Indian children aged 9 - 24 months

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Objectives. To investigate the safety of live attenuated varicella vaccine (Oka strain) and the optimal virus titre/dose required for immunogenicity in healthy South African children.

Design. Double-blind randomised clinical study using two different lots of varicella vaccine, each at two different titres. Subjects were randomly allocated to groups 1, 2, 3 and 4 to receive vaccine containing a mean virus titre of $10^{4,5}$, $10^{3,1}$, $10^{3,9}$ and $10^{2,7}$ PFUs per dose respectively. Clinical signs and symptoms were followed up for 42 days post-vaccination. Specific varicella antibodies were measured by an indirect immunofluorescence method in sera obtained on day 0 and day 42.

Setting. City Health Clinic, Chatsworth, Durban.

Participants. A total of 200 healthy 9 - 24-month-old children were vaccinated, of whom 189 (44,5%) completed the study.

Main outcome measures. Pre- and post-vaccination varicella antibody levels. Adverse events following varicella vaccination.

Results. The vaccine was safe and well tolerated. No local symptoms were reported. Skin reactions were specifically solicited in this study: 21 reactions were reported in 8,5% (17/200) of children. Vesicles were reported in 2 vaccines (≤ 10 vesicles in both cases). One serious adverse event was reported: hospitalisation for bronchopneumonia on day 16 post-vaccination which resolved without sequelae. Around day 42 post-vaccination (range 35 - 63 days) all the 176 initially seronegative subjects had seroconverted for varicella antibodies. Post-vaccination geometric mean titres (GMTs) were 104,1, 66,2, 69,5 and 77,0 for groups 1 - 4 respectively. Six subjects who were initially seropositive maintained or increased their titres post-vaccination; 3 of the 6 showed a booster response (a ≥ 4-fold increase from the pre-vaccination titre).

Conclusions. Varicella vaccine was found to be safe, immunogenic and well tolerated. No difference in seroconversion rates or GMTs, either between groups receiving the two vaccine lots or between groups receiving the different titres of each lot, was shown.

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The inclusion of different vaccines in routine vaccination schedules is under serious scrutiny as new vaccines become available. A live attenuated varicella vaccine (Oka strain) was developed in Japan in the early 1970s and is currently being used in many countries for the prevention of varicella in high-risk and immunocompromised children.[1] Although not registered in South Africa, the vaccine (Varilrix; SmithKline Beecham Biologicals) is available for the above purpose and has been used in Belgium and other European countries since 1984.

In Japan and South Korea, the same Oka strain is used in a vaccine licensed for general vaccination of all healthy children at an early age, and more than 1 million doses have been administered since 1986.

In South Africa, chicken-pox is not a notifiable disease, and there is therefore a paucity of morbidity and mortality data. The disease is widely recognised to be present, although the vast majority of cases are not hospitalised. In a study of 244 healthy adults in South Africa, the overall level of immunity to varicella was found to be 90%.[2]

The inclusion of varicella vaccine in routine vaccination schedules in South Africa would result in the protection of both healthy and high-risk children and have considerable advantages. These include prevention of the complications of varicella (e.g. encephalitis, pneumonia, scars); inhibition of the spread of the disease, particularly in institutions; a possible reduction of the incidence and/or severity of zoster in later life; and cost-benefits of reduced morbidity in the population, e.g. the need for a working parent to remain home with a sick child.

Varicella vaccine has not been evaluated previously in South Africa. The present study was undertaken to evaluate in healthy babies aged 9 - 24 months the safety and immunogenicity of two different lots of varicella vaccine and to evaluate the safety and immunogenicity of a 'fresh' vaccine (soon after manufacture) and a vaccine at a lower titre (artificially obtained by exposure at 37°C for 7 - 10 days). The latter was thought to mimic prolonged storage conditions at refrigerator temperatures, since continuity of a 'cold chain' is not always maintained in developing countries, and may result in loss of potency of heat-sensitive live vaccines.

Subjects and methods

The vaccine

Varilrix was the live attenuated varicella vaccine (Oka strain) used in this study. Two untreated 'fresh' vaccine lots (VA 101

A42 and VA 104 B42) were used, as well as vials of these two lots exposed at 37°C for 10 and 7 days respectively (which reduced the initial virus titre). The mean virus titres/doses (PFUs) received by subjects in groups 1 - 4 respectively were $10^{4,5}$ (high titre), $10^{3,1}$ (low titre), $10^{3,9}$ (high titre) and $10^{2,7}$ (low titre). The vaccines were supplied in monodose vials, each was reconstituted with an individual ampoule of water for injection and one 0,5 ml dose was administered subcutaneously into the left upper arm.

Subjects

The subjects were 200 healthy Indian boys and girls 9 - 24 months (mean 15,9 months) residing in Chatsworth, Durban. Exclusion criteria included history of clinical varicella infection, recent exposure to varicella or zoster, recent administration of immunoglobulins, adverse reactions to previous vaccination, or other vaccination (except oral polio) within 4 weeks. Prior to enrolment, children underwent clinical examination and written informed consent was obtained from the parent or guardian. Subjects were randomly allocated to 1 of 4 groups (groups 1, 2, 3, 4) in the order in which they were enrolled in the study. One hundred and eighty-nine of the 200 subjects (94,5%) completed the study.

Study design

The study was approved by the Ethics Review Committee of the Faculty of Medicine, University of Natal. On day 0, subjects underwent physical examination, axillary body temperature was recorded, a pre-vaccination blood sample was collected and one dose of the vaccine was administered. Parents were asked to observe the child for any clinical signs or symptoms and were instructed to contact the investigator immediately if any rash/eruption or serious adverse event occurred. All parents were contacted on day 21 to evaluate the presence of clinical signs and symptoms. On day 42, a physical examination took place, a post-vaccination blood sample was collected and the study period was reviewed for any signs or symptoms. Any medication received during the study period was also recorded.

Serology

Separated pre- and post-vaccination serum samples (day 0 and day 42) were stored at –20°C. Specific varicella antibodies were measured by an indirect immunofluorescence method.[3] Samples which showed no fluorescence or barely visible fluorescence at the starting dilution (1/4) were considered seronegative. The titre was expressed as the reciprocal of the highest dilution still showing a varicella/zoster virus-associated fluorescence.

Seroconversion was defined as the appearance of antibodies in the serum of subjects who were seronegative before vaccination (i.e. titre < 4 prior to vaccination to ⩾ 4 in post-vaccine serum). A booster response was defined as a fourfold increase in a positive pre-vaccination titre. Geometric mean titres (GMTs) of specific varicella antibodies were calculated in post-vaccination sera in seroconverters and in pre- and post-vaccination sera in the initially seropositive subjects. GMTs were calculated by using the log transformation of titres ⩾ 4 and then taking the antilog of the mean of these transformed values. Analysis was performed by SAS using a type I error (alpha) of 5%.

Immunogenicity

GMTs of specific varicella antibodies were calculated for each group at the second time point of the study (day 42) in seroconverters. Ninety-five per cent confidence intervals of these GMTs were calculated. The percentage of seroconverters was calculated for each group as defined above. Ninety-five per cent confidence intervals of these percentages were calculated.

Safety and reactogenicity

Skin reactions were solicited and the distribution and the number of papules and/or vesicles observed were described. Maculopapular rash, macular rash and papular rash were grouped together as papular rashes, and papulovesicular rash and vesicular rash were grouped together as vesicular rashes. The observed unsolicited clinical signs and symptoms were described and the percentage of subjects reporting local and general symptoms calculated for the follow-up period.

Results

Immunogenicity of the vaccine

A total of 182 subjects were included in the immunogenicity analysis, of whom 176 were seronegative and 6 were seropositive before vaccination. Eighteen subjects were eliminated from the analysis: 6 for irregular schedule of visits and 12 for a missing blood sample.

Table I shows the seroconversion rates and GMTs of specific varicella antibodies at day 42 post-vaccination for the 176 initially seronegative subjects. All these children had detectable antibody titres post-vaccination. GMTs varied from 66,1 to 104,1 among the four groups. Children below 18 months of age had GMTs (74; (95% CI: 64,0 - 85,0)) comparable to those of children over 18 months (91; (95% CI: 71,2 - 115,1)) (P = 0,144).

Table I. Percentage seroconversion and GMT of varicella antibodies on day 42 post-vaccination in initially seronegative subjects

Group*	No.	% sero-conversion	GMT (titre range)	CL 95%† lower	CL 95%† upper
1	47	100,0	104,1 (16 - 1 024)	84,0	129,0
2	42	100,0	66,1 (8 - 512)	47,8	91,5
3	42	100,0	69,5 (8 - 512)	54,4	88,9
4	45	100,0	77,0 (32 - 512)	64,3	92,2

* Group 1, 2, 3 and 4 received vaccine containing $10^{4,5}$, $10^{3,1}$, $10^{3,9}$, $10^{2,7}$ PFUs of varicella virus per dose respectively.
† Lower and upper 95% GMT confidence limits.

Six infants (2 each from groups 2, 3 and 4) were initially seropositive for specific varicella antibodies. All 6 children maintained or increased their titre post-vaccination. There were 3 booster reactions (defined as a fourfold or greater increase in pre-vaccination titre), 2 in group 2 and 1 in group

Table II. Skin reactions reported after vaccination

Skin reactions	Vaccine groups 1 (*N* = 50)	2 (*N* = 51)	3 (*N* = 50)	4 (*N* = 49)	1 - 4 (*N* = 200)
Papular rashes*	2	2	4	1	9
Vesicular rashes*	1			1	2
Other skin reactions*	2	2	5	2	10
Any skin reaction†	4 reactions in 2 subjects	4 reactions in 4 subjects	9 reactions in 8 subjects	4 reactions in 3 subjects	21 reactions in 17 subjects

* Figures indicate the number of times a reaction was reported.
† $P = 0,21$ (Fisher's exact test comparing number of subjects with skin reaction in each group).

4. Their post-/pre-titre ratios were 16, 64 and 16 respectively.

Reactogenicity

Signs and symptoms of any kind were reported during the 42-day follow-up period in 22,5% of subjects (46/200) — 22% in group 1, 19,6% in group 2, 28% in group 3 and 20,4% in group 4; all reported symptoms were general, and no symptoms were reported at the injection site.

Skin reactions were specifically solicited in this study. Twenty-one skin reactions were reported in 8,5% of subjects (17/200): 4%, 7,8%, 16% and 16,1% in groups 1 - 4 respectively. Vesicles were reported in only 2 subjects (10 vesicles or fewer in both cases): 1 in group 1 and 1 in group 4. Mild, attenuated chicken-pox was clinically diagnosed in the subject from group 4 on day 16; fever and bilateral conjunctivitis were also reported in this subject. He was initially seronegative and had a post-vaccination titre of 32. Details of reported skin reactions are given in Table II.

Sixty-three unsolicited symptoms (including fever, gastro-intestinal disorders, respiratory infections, measles and conjunctivitis) were reported in 20,5% (41/200) of subjects: 20% in group 1, 21,6% in group 2, 16% in group 3 and 24,5% in group 4. One symptom was assessed by the investigator as 'possibly related' (fever in a subject in group 4). Only one serious adverse event was reported: bronchopneumonia in a subject in group 2 on day 16 post-vaccination; he was hospitalised and recovered without sequelae. This event was considered to be unrelated to varicella vaccination.

Discussion

Varicella vaccine (Oka strain) has been given to over 10 000 healthy individuals and patients with a variety of diseases in well-controlled trials held since the 1970s. They showed that the Oka strain was attenuated, stable and tended not to return to virulence, that all the vaccines gave a similar high level of immunogenicity, and that all were equally well tolerated.[3,4] Despite this, varicella vaccine is not in routine use in most countries. The reason for this is that certain lots of the vaccine given to leukaemic children resulted in a high incidence of often severe vaccine-associated chicken-pox.[5] These lots were subsequently found to contain 'less attenuated' virus which may have resulted in an altered host immune response. Clinical trials with new lots have since been undertaken and licensing and widespread use of the vaccine are now imminent in the USA and several European countries.

Another major concern about varicella vaccine has been whether there would be an increased risk of shingles (zoster) in immunised children. Studies in the USA have shown that the rate of zoster was lower than that expected after natural chicken-pox in both healthy and leukaemic children.[5,6]

The primary objective of the present study was to evaluate the immunogenicity and reactogenicity of two different lots of varicella vaccine and to compare their immunogenicity at two different potencies; release titre (high: $10^{4,5}$ - $10^{3,9}$) and expected expiry titre (low: $10^{3,1}$ - $10^{2,7}$), i.e. a range of 31 623 - 501 PFUs/dose. Vaccine doses containing 500 to 1 000 PFUs, have been shown to be immunogenic in > 90% of children and adults.[3] Reviews of varicella vaccine trials involving vaccines ranging from 400 to 30 000 PFUs per dose showed similar high levels of immunogenicity and safety for all the vaccines and indicated that the attenuated Oka strain is phenotypically and genetically stable. There was no evidence of any tendency to revert to virulence; however, lower rates of seroconversion were seen with lower doses of vaccine.

This study provides sufficient evidence that this varicella vaccine is safe, immunogenic and well tolerated in young South African children, as was found to be the case in similar studies with several Oka strain vaccines in Japan, the USA and Turkey.[1,7,8]

The results of this study confirm findings elsewhere that the rate of seroconversion is generally greater than 95% in healthy subjects after one dose of varicella vaccine.[3,5,7] The 200 children aged 9 - 24 months who received one subcutaneous injection of this Oka strain varicella vaccine showed excellent tolerance of the vaccine and all initially seronegative subjects had seroconverted for varicella antibodies by day 42. Only 6 subjects (3%) in the study population were seropositive for varicella before vaccination and they were over 12 months of age. Of the 24 other subjects who were younger than 12 months of age, all were initially seronegative for varicella antibodies. These data suggest that most might have lost any maternally derived immunity by the time they were 9 - 12 months old. At this early stage, when the majority of infants are seronegative and therefore likely to be susceptible to varicella, the vaccine used was thus shown to be safe, well tolerated and to elicit an extremely satisfactory immune response.

Varicella vaccine appears to be highly effective in protecting healthy children against infection. Mild breakthrough cases of varicella occasionally occur months or years after vaccination,[9] but the vaccinees are indeed

protected against varicella cases of clinical relevance. During the more than 15-year follow-up of vaccinated children in Japan and the 7-year follow-up of those in the USA, the protection conferred by the vaccine appeared to be long-lasting.[1,8] The duration of protection needs, however, to be examined further before the need for booster doses can be assessed properly.

Live vaccines are heat-sensitive and may therefore be subject to loss of potency following breaks in the 'cold chain', a common occurrence in developing countries. Different vaccine viral titres were used to investigate the response to lower titres obtained after exposure at 37°C for several days produced a titre mimicking prolonged storage conditions in a refrigerator. The two vaccine lots as well as the different titres used in this study all induced 100% seroconversion. These results allow for less stringent storage conditions than previously recommended (i.e. refrigerator as compared to freezer) for this vaccine. The stability of this vaccine compared with the currently available commercial varicella vaccine has been demonstrated in 'real time stability' *in vitro* studies with vaccine stored at 4°C; the stability was not demonstrated as such in this study, however.

Preliminary studies have shown that varicella vaccine is safe in adults and in both healthy and leukaemic children, with few reported adverse reactions.[1,3,4] The only significant reactions which have been described elsewhere and these only rarely (in 5 - 10% of vaccinees) are mild fever and skin rashes with occasional vesicles by no means comparable in number and distribution to those seen during natural chicken-pox.

In our study, skin reactions were reported in 8,5% of subjects (Table II). Most of these were mild maculopapular rashes (1 - 10 spots). Skin reactions were not reported at significantly different rates between groups or titres ($P = 0,21$; Fisher's exact test). Only 2 children had vesicles (10 or fewer in both cases): 1 of these 2 was clinically diagnosed with a mild, attenuated chicken-pox that appeared 17 days after vaccination with fever and bilateral conjunctivitis; however, her post-vaccination titre was only 32 which is very low when compared with titres (generally ≥ 512) induced by natural varicella in non-vaccinees.

No parent or guardian reported any local sign or symptom at the injection site and three-quarters of them did not mention any symptoms at all during the 42-day study period. Among those who did, the symptoms were mild and distributed almost equally in the 4 groups of vaccinees. The fact that the incidence of severe post-vaccination events following varicella vaccine is minimal further justifies the prevention of this relatively mild childhood disease by vaccination.

In a recent conservative cost-benefit analysis in the USA, routine varicella vaccination at US$35/dose has been shown to be cost-effective for both healthy and leukaemic children when given as a combined vaccine with MMR as part of the routine vaccination schedule.[10] As a follow-up to this study, our results allow for further studies to investigate the possibility that varicella vaccine could be simultaneously administered with measles vaccine since, in South Africa, the latter is routinely administered to infants aged 9 - 10 months, and again at 15 months (mean age of infants in this study).

The South African National Advisory Group on Immunisation has recently determined infectious disease immunisation priorities in this country to be hepatitis B and *Haemophilus influenzae* type B followed by the pneumococcal pneumonias. While not a priority at present, when financial resources become available, routine varicella vaccination of all children in South Africa will have considerable advantages. Although most varicella infections are benign and serious morbidity is infrequent, the disease is highly contagious and leads to interruption of children's attendance at school and disturbs the organisation at créches and preschools. The necessity for home care can have significant effects on the parents' working life, and routine vaccination is therefore likely to be cost-effective. Other advantages of varicella vaccination include the prevention of complications (pneumonia, encephalitis, scars), inhibition of the spread of institutional outbreaks and thus the diminishing of exposure of high-risk subjects to the disease; a possible reduction in the occurrence and/or severity of zoster in later life[10-12] is also likely. Although not registered in South Africa at present Varilrix is currently being used in many countries for the prevention of varicella in high-risk and immunocompromised children.

General varicella vaccination of South African children seems an attractive prospect to prevent the normal morbidity of chicken-pox; however, until finances become available, we recommended that it be administered routinely to 'high-risk' non-immune children (e.g. those with malignant disease) and to institutionalised children.

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