Safety and Immunogenicity of a Combined Live Attenuated Measles, Mumps, Rubella, and Varicella Vaccine (MMR\textsubscript{II}V) in Healthy Children

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An investigational tetravalent combined measles, mumps, rubella, and varicella vaccine and measles-mumps-rubella and varicella vaccines at separate injection sites given at the same visit were evaluated with respect to safety and cell-mediated and humoral immune responses at 6 weeks and 1 year after vaccination. Varicella seroconversion rates and lymphocyte proliferation responses were 100% for both vaccine groups at 6 weeks and 1 year. However, the antibody titer to varicella was lower in the combined vaccine group at 6 weeks, but there was no statistical difference in cell-mediated immune responses. One-year geometric mean titers were not statistically different. Seroconversion rates for measles, mumps, and rubella were 100% for both vaccine groups at 6 weeks and 1 year. Long-term follow-up of these immune responses is planned.

The recent licensure of the live attenuated Oka strain of varicella vaccine (Varivax; Merck Sharp & Dohme, West Point, PA) focused continued interest on the combination of varicella vaccine with other childhood vaccines. The addition of varicella vaccine to the current measles-mumps-rubella combination would be desirable in the routine immunization of healthy children. A combined vaccine would be acceptable to patient, parent, and practitioner, promoting universal immunization. Previous studies have evaluated different combinations of measles, mumps, rubella and varicella vaccines with promising results [1–9].

The work reported here was a single-blind study evaluating safety and humoral and cellular immune responses at 6 weeks and 1 year after vaccination with an investigational tetravalent combined vaccine A (measles-mumps-rubella) and vaccine B (varicella) (vaccine AB, group 1) developed by Merck Research Laboratories (MRL) and vaccines A and B (vaccine A + B, group 2) given at separate injection sites at the same visit.

Methods

Study design. Between November 1992 and March 1993, 111 healthy children, with a mean age of 15.7 months (range, 12–19; 56 girls, 55 boys), with no history of naturally acquired measles, mumps, rubella, or varicella-zoster virus (VZV) infection were recruited from suburban pediatric practices in the Philadelphia area. Vaccinees were randomized in a single-blind fashion into 2 groups. Group I included 57 children who received the combination vaccine in one arm and a placebo in the other. Group 2 was 54 children who received separate simultaneous injections of vaccine A in one arm and vaccine B in the other.

Blood for determination of cellular and humoral immune responses was drawn before vaccination (day 0) and at 6 weeks and 1 year after vaccination. Local and systemic reactions were recorded for the 42-day period following each immunization. Study personnel were notified of any rash, fever, or unusual reaction as described [2]. At the 6-week and 1-year visits, exposure histories and details of breakthrough cases of measles, mumps, rubella, varicella, and herpes zoster were obtained.

Vaccine. Vaccine B contained 3625 pfu of varicella virus. Combination vaccine (vaccine AB) contained 3785 pfu of VZV. The measles-mumps-rubella component of the combined vaccine was similar to the currently marketed vaccine (Merck Sharp & Dohme). Vaccine was stored in the lyophilized form at -20°C and reconstituted immediately before use.

Humoral immunity. Antibodies to VZV were measured at MRL using a glycoprotein ELISA previously described [10]. A positive result was a calculated end point titer $>0.625$.

Antibodies to measles, mumps, and rubella viruses were measured by a secondary ELISA developed at MRL. Titters $>5.0$, $>2.0$, and $>8.0$ U were considered positive for measles, mumps, and rubella viruses, respectively.

Lymphocyte proliferation assay. Lymphocyte proliferation (LP) assays for VZV-specific cell-mediated immunity (CMI) were done as described [11] with one modification: Two dilutions of antigen (either 1:100 and 1:200 or 1:200 and 1:400, depending on antigen content) were used to determine maximal CMI responses. A result was considered positive if the stimulation index (SI or counts per minute in VZV antigen–stimulated cultures/counts per minute in control antigen–stimulated cultures) was $>3.0$.

Statistical analysis. All ELISA titers were logarthimically transformed to obtain geometric mean titers (GMTs). GMTs and LP responses were analyzed using a t test.
Results

Vaccine Safety

All doses of vaccine were generally well tolerated. There were 9 measles-like rashes (8.1%) similar to those seen after administration of licensed vaccine: 6 in group 1 and 3 in group 2. The measles-like rashes had a mean duration of 2.9 days (range, 2–6). There were 5 varicella-like rashes (4.5%): 2 in group 1 and 3 in group 2. Rashes were mild and characteristic of previously observed varicella vaccine rashes, with a median of 2 lesions and mean duration of 4.2 days (range, 1–8). Fever was observed in 7 (6.3%) of 111 vaccinees after vaccine administration. Four vaccinees with a measles-like rash had associated fever of ≥39.2°C.

VZV-Specific Immune Responses

VZV-specific humoral responses. For the 97 vaccinees who were seronegative for VZV antibody or had an SI <3 before immunization (or both), humoral responses were analyzed. Varicella seroconversion rates 6 weeks after vaccination were 100% for both groups of initially seronegative children. The GMT for VZV in group 2 at 6 weeks was significantly higher (15.3) than that in group 1 (7.0; \(P = .0001, t\) test; table 1). However, the GMT 1 year after immunization was not statistically different between the 2 groups. Persistence of antibody at 1 year for all evaluable vaccinees was 100%.

VZV-specific LP responses. Fifty-three (55%) of the 97 evaluable vaccinees had VZV-specific LP responses determined. The mean SIs at 6 weeks and 1 year for both groups were similar (table 1).

Immune responses after exposure to varicella. One year after vaccination, 22 vaccinated children had known exposures to varicella. Four children had household and 18 had nonhousehold exposures, with 13 exposures lasting >4 h. No breakthrough cases of varicella occurred after exposure. Data from the 22 exposed children were included in the 1-year analyses because results were similar when data were analyzed including or excluding the exposed subjects.

Antibody Responses to Measles, Mumps, and Rubella

Measles. Of the 111 children tested on day 0, 103 (93%) were seronegative for antibody to measles. Seroconversion rates 6 weeks after vaccination were 100% for both groups of seronegative children, with no significant difference in the titers between the 2 groups (\(P > .5, t\) test; gp1 GMT, 88.7 [SD 2.0]; gp2 GMT, 71.9 [SD 2.2]). The 1-year GMTs were 141.0 (SD 24.6) for group 2 and 140.6 (SD 2.3) for group 1.

Mumps. Of the 111 vaccinees, 107 (96%) had negative mumps titers on day 0. Seroconversion rates by 6 weeks after vaccination were 100%, with similar 6-week (gp1 GMT, 87.0 [SD 3.3]; gp2 GMT, 87.4 [SD 2.8]) and 1-year (gp1 GMT, 76.2 [SD 2.7]; gp2 GMT, 82.4 [SD 3.8]) titers for both groups.

Rubella. Of the 111 participants, 110 (99.1%) were seronegative for rubella antibody on day 0. Seroconversion rates were 100% at 6 weeks for both groups, with similar 6-week (gp1 GMT, 100.3 [SD 2.5]; gp2 GMT, 80.0 [SD 2.4]) and 1-
Table 2. Characteristics in published studies combining measles, mumps, rubella, and varicella vaccines in healthy children.

<table>
<thead>
<tr>
<th>Reference (year)</th>
<th>Type of study</th>
<th>No. enrolled, (age in months)</th>
<th>Varicella vaccine dose, pfu</th>
<th>Varicella seroconversion rate, %* (time after vaccination)</th>
<th>Varicella seropositivity rate, % (time after vaccination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6, 7] (1985, 1986)</td>
<td>Separate simultaneous vaccine A (SmithKline) + V vs. mixed simultaneous vaccine A (SmithKline) + V varied varicella doses; booster diphtheria, tetanus, and oral polio administered</td>
<td>205 (15–24)</td>
<td>1100, 4300, 6000, 12,000</td>
<td>42, 79, 91, 95 (not indicated)</td>
<td>ND</td>
</tr>
<tr>
<td>[8] [1, 2] (1986)</td>
<td>Combined MMRV</td>
<td>24 (15)</td>
<td>Not indicated</td>
<td>96 (4 w)</td>
<td>ND</td>
</tr>
<tr>
<td>[3]</td>
<td>Mixed simultaneous vaccine A (SmithKline) + V; varied varicella, measles, mumps doses</td>
<td>200 (14–23)</td>
<td>1800, 5400</td>
<td>77, 98 (8 w)</td>
<td>ND</td>
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<tr>
<td>[4]</td>
<td>Combined MMRV vs. separate vaccine A (Merck) followed 3 mo later by V or vaccine A (Merck), respectively</td>
<td>206 (12, 15)</td>
<td>950 (vaccine A + V), 2300 (MMRV)</td>
<td>74, 95 (3, 6, or 9 mo)</td>
<td></td>
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<tr>
<td>[5]</td>
<td>Separate simultaneous vaccine A (Merck) + V, followed 6 w later by placebo (PI) vs. vaccine A (Merck) + PI followed 6 w later by V</td>
<td>111 (15–24)</td>
<td>6900</td>
<td>99</td>
<td>ND</td>
</tr>
<tr>
<td>[9]</td>
<td>Mixed simultaneous vaccine A + V (2 dosages) vs. separate vaccine A followed by vaccine A (SmithKline)</td>
<td>196 (13–17)</td>
<td>2000, 5300</td>
<td>72, 97 (80 ± 4 d)</td>
<td>ND</td>
</tr>
<tr>
<td>Current report</td>
<td>Combined MMRV vs. separate simultaneous vaccine A (Merck); CMI responses</td>
<td>111 (12–19)</td>
<td>3625 (vaccine A + V; group 2), 3785 (MMRV; group 1)</td>
<td>100, 100 (6 w)</td>
<td>100, 100 (1 y)</td>
</tr>
</tbody>
</table>

NOTE. d = days; w = weeks; mo = months; y = years; vaccine A = measles-mumps-rubella (with manufacturer); MMRV = combined measles, mumps, rubella, and varicella vaccine; V = varicella vaccine; CMI = cell-mediated immune responses; ND = not done.

* Measles, mumps, and rubella seroconversions ranged from 77% to 100%, depending on study.

Discussion

The frequency of local and systemic reactions was similar between the combined and separate vaccine groups. In the present study, 100% seroconversion to all four viral components occurred by 6 weeks after vaccination and persisted 1 year from vaccination. CMI responses to varicella vaccine were comparable for both groups at 6 weeks and 1 year.

This study extends the results of previously published studies of combining measles, mumps, rubella, and varicella vaccines in healthy children, which are summarized in table 2 [1–9]. Differences in study design, sample size, vaccine dosage, and vaccine source were the main distinguishing features between the studies. Lower seroconversion rates for varicella were attributed to the direct mixing of measles, mumps, rubella, and varicella vaccines in the same syringe [3, 6, 7, 9]; lower doses of varicella vaccine [4, 6, 7]; and differences in levels of varicella virus attenuation [4].

Our study noted 100% varicella seroconversion in both groups. However, the statistically higher GMT (14.65) in group 2 compared with the lower GMT (7.2) in group 1 at 6 weeks may indicate interference on the extent of VZV replication in the host when all four viruses are injected in the same location (measles, mumps, rubella, and varicella). Whether there was a direct local site or more “systemic” viral vaccine interaction is not clear. This is the first reported study to analyze VZV-specific CMI responses in measles, mumps, rubella, and varicella vaccine recipients. Positive CMI responses were induced...
in all vaccine recipients and persisted at 1 year after vaccination. No statistically significant difference was noted between the 2 groups.

Varicella vaccine does not appear to interfere with measles, mumps, or rubella seroconversions as indicated by this and previously published studies (table 2) [1–9]. Seroconversion rates were similar at all time points tested for measles, mumps, and rubella in the described studies. The present study found similar titers to measles, mumps, and rubella antigens in both vaccine groups at 6 weeks and 1 year after vaccination.

In summary, humoral seroconversion rates, CMI responses, the persistence of immune responses at 1 year, and the frequency of local and systemic reactions after administration of a combined vaccine are comparable results obtained after the separate administration of measles, mumps, rubella, and varicella vaccines. This cohort of children will have long-term follow-up to evaluate the persistence of both humoral and cell-mediated immunity over time. Further studies are planned to incorporate a better stabilizer and higher titers of varicella vaccine virus in the hope of developing a refrigerator-stable combined vaccine that will elicit antibody titers to VZV similar to those seen after vaccination with monovalent varicella vaccine.

Acknowledgments

We thank Drexel Hill Pediatrics, Main Line Pediatrics, and the participating children and parents. We also thank Stuart Starr, Sharon Piercy, Nancy Tustin, William Hurni, Beth Arnold, Wendy Brockett, Beverly Rich, Paul Keller, and David Olaleye for clinical and laboratory assistance.

References