A randomized trial demonstrating successful boosting responses following simultaneous aerosols of measles and rubella (MR) vaccines in school age children

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Received 1 October 2001; received in revised form 27 February 2002; accepted 11 March 2002

Abstract

The reactogenicity and immunogenicity of combined measles and rubella (MR) booster vaccination, via aerosol and subcutaneous routes, was assessed in 562 healthy children. Rates of rubella seroconversion and geometric mean titers (GMT) were similar for both routes. Rates of measles PN seroconversion, GMT and measles ELISA post-vaccination seropositivity and seroconversion rate were each higher for aerosol vaccine (54%, 3928 IU/l, 99.6 and 98.8%) than for subcutaneous vaccine (7%, 866 IU/l, 92.2 and 82.4%) (P < 0.01). Reactogenicity was higher for subcutaneous vaccine (P < 0.05). This study demonstrates that aerosol vaccine was more immunogenic for measles antibodies, and equally immunogenic for rubella antibodies. Aerosol vaccine was less reactogenic. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Measles; Rubella; Aerosol administration

1. Introduction

Aerosol administration of measles vaccines in mass campaigns was first proposed by Dr. Albert Sabin [1]. Factors favoring the aerosol route include its non-invasiveness, documented immunogenicity in seronegative and seropositive children, potential to stimulate local respiratory tract immunity and prevent reinfection, better acceptance by the population as it does not require parental injection, reduction in the risks of cross-infection entailed by parental application, lower cost, and easy administration by non-medical personnel [2]. Aerosol administration of measles vaccine has been used in mass campaigns in Mexico, being administered both as primary and booster dose to nearly 4 million children, with fewer side effects reported than those usually observed after subcutaneous vaccination [3]. Recently, studies have shown that aerosol administration of Edmonston-Zagreb (EZ) measles vaccine is more immunogenic than subcutaneous administration of this same vaccine or of Schwarz measles vaccine at 1 month and 1 year after vaccination [4]. Antibody titers continued to be substantially higher for the aerosol group at 2 years after vaccination [5].

Immunogenicity of rubella vaccine by aerosol has been demonstrated by Ganguly et al. [6,7]. Its combined administration with measles vaccine intranasally has proved to have been immunogenic in small-scale studies [8]. The advantages of mass campaigns with combined vaccine include the possibility of increasing population immunity to measles, while simultaneously achieving primary vaccination for rubella in many children in developing countries where neither measles and rubella (MR) nor MMR vaccines are routinely given. Indeed, the combination of MR vaccines in campaigns targeted towards measles elimination/eradication has previously been endorsed [9,10]. Although most immunization programs in developed countries, and in some developing countries, are using combined vaccines (measles, mumps, rubella), both for primary and for booster doses [11], the addition of mumps vaccine adds considerably to the cost of MR vaccine [12], thus making MMR less attractive for mass campaigns in many developing countries.

In the present study, we describe the results of a randomized controlled study in which we compare the immunogenicity and frequency of clinical symptomatology after...
subcutaneous and aerosol administration of combined rubella and measles vaccine to school age children.

2. Methods

2.1. Design

Study was conducted in eight municipalities in the state of Hidalgo, Mexico. No cases of measles had been reported in these communities since 1994. Coverage of measles primary vaccination was approximately 99%. Children enrolling in first grade in selected schools, healthy, not having been vaccinated with measles or rubella booster doses, and with no previous meases were recruited between 5 October and 16 December 1998. Clinical study and physical examination, including weight and height were performed.

A listing including all public schools in the area was elaborated. Individual schools were randomized to receive one of the eight open label vaccines. In this article, we describe two arms: subcutaneous, lyophilized EZ measles and RA27/3 rubella vaccine (Swiss Serum and Vaccine Institute) and aerosol liquid EZ and RA27/3 MR vaccine (Swiss Serum and Vaccine Institute).

The study, as designed, had a power of 80% to detect 2.5% differences in seroconversion frequency between arms (two-sided α level, 0.05). The randomization unit was the participating school. Randomization was accomplished by use of a table of random numbers. After eligibility determination, informed consent was requested from parents or tutors. Approval by appropriate institutional review boards was obtained.

Primary endpoint measures were MR antibodies, measured by ELISA and plaque neutralization (measles) and ELISA (rubella). Secondary endpoint was the frequency of symptoms during the 14 days after vaccination.

2.2. Vaccines

Lyophilized EZ and RA27/3 MR vaccine (Moru-Viraten) for subcutaneous use was provided by the Swiss Serum and Vaccine Institute, Berna, Switzerland. The EZ component contained 3.9 log10/ml plaque forming units (pfu) and the RA27/3 component contained 4.7 log10/0.5 ml tissue culture infective dose (TCID) 50 (lot 148410101). The vaccine was provided in individual doses (0.5 ml), which were reconstituted with the diluent provided by the manufacturer. Vaccines were kept at 4–6 °C until use.

Liquid MR vaccine for aerosol administration was shipped by Swiss Serum and Vaccine Institute, (lot IX A2) frozen in liquid nitrogen. The EZ component contained 3.9 log10/ml plaque forming units (pfu) and the RA27/3 component contained 4.7 log10/0.5 ml tissue culture infective dose (TCID) 50 (lot 148410101). The vaccine was provided in individual doses (0.5 ml), which were reconstituted with the diluent provided by the manufacturer. Vaccines were kept at 4–6 °C until use.

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2.3. Subcutaneous administration of vaccine

Lyophilized vaccine was diluted with 0.5 ml of the diluent before administration. Vaccines were administered using aseptic procedures in the deltoid area of the left arm of participants, using a 27 mm × 13 mm gauge syringe.

2.4. Aerosol administration of vaccine

The aerosol method was designed by Dr. Fernandez de Castro and has been described previously [4]. This method has been used in mass campaigns in Mexico [3]. Vaccine vials were quickly unfrozen before administration using water at room temperature, and used for up to 40–45 vaccinations. A plastic nebulizer (AeroMist Treatment Set of IPI Medical Products, catalogue no. 4107, IPI Medical Products) containing the vaccine was then placed in a container with crushed ice, and aerosols were generated by connecting the nebulizer to an electric 12 W compressor providing approximately 0.1 ml of aerosol in 30 s bursts. Vaccine was administered through a plastic cone joined to the nebulizer by a plastic tube. A paper cone placed on the plastic cone loosely fitted over the nose and mouth of the child, and was discarded after each use.

2.5. Adverse effect follow-up

Children were monitored by the schoolteachers, who were required to complete a structured questionnaire providing information on clinical symptoms or school absences of the participants for 14 days after vaccination. When a child was absent from school, field workers visited the child’s home to corroborate health status.

2.6. Laboratory techniques

Venous blood samples were drawn at 0 and 120 days, between 2 February and 3 June 1999. Measles IgG were determined by indirect ELISA, Measles Section, Centers for Disease Control (CDC), Atlanta, GA [13,14]. Thirty pairs of samples from each group were randomly tested for plaque reduction neutralization (PRN) test on baseline and 6-month post-vaccination samples, as previously described by Albrecht et al. Results were reported in IU/l [15,16]. Titers less than 120 IU/l were considered seronegative [17]. Rubella IgG was determined by Enzygnost Anti-rubella Virus/IgG (Dade Behring) [18].

2.7. Analysis

Nutritional index was calculated according to the weight for height index, as recommended in the official
Mexican Norm [19]. Rubella seropositivity was defined as titters \( \geq 15 \) IU/ml. Rubella seroconversion was defined as a change from seronegative to seropositive (\(<15\) IU/ml) or a four-times rise in the level of pre-vaccination (\( >15\) IU/ml). Measles seroconversion by ELISA was defined as a change from seronegative or indeterminate to seropositive. Measles seropositivity was defined as titters \( \geq 120\) IU/l. Measles seroconversion by PN was defined as a change from seronegative to seropositive (\(<120\) IU/l) or a four-times rise in the level of pre-vaccination (\( >119\) IU/l). Geometric means were calculated for the titters of antibodies. Categorical variables were compared using \( \chi^2\)-test or Fisher exact test and normally distributed continuous variables with the \( t\)-test. Univariate 95% confidence intervals were calculated. Chi-square test was used to compare seroconversion rates, seropositivity rates and adverse reactions among the groups. Logistic regression multivariate analysis was used to determine relative risks associated with seroconversion. DBASE IV and STATA 5.0 programs were used for data analysis.

### 3. Results

Initial selection of the eight-arm study included 5251 children from 23 schools; 2056 did not meet eligibility criteria (main reason for non-eligibility was having already received measles vaccine booster dose); 959 refused to participate; 2236 children were initially enrolled. Of these 596 were enrolled in the two arms described here (326 received the subcutaneous EZ and RA27/3 MR vaccine and 270 received the aerosol liquid EZ and RA27/3 MR vaccine). Of these, 21 were lost to follow-up (11 to the subcutaneous arm and 10 to the aerosol arm) and 13 refused to donate the second blood sample (eight from the subcutaneous arm and five from the aerosol arm). Therefore, 562 donated the post-vaccination serum specimen (307 from the subcutaneous arm and 255 from the aerosol arm) and were included in the analysis. Table 1 shows the baseline characteristics of study population. The aerosol group had a significantly higher proportion of children with positive measles ELISA titters, although pre-vaccine neutralization antibodies were similar (Table 1).

#### 3.1. Acute adverse reactions

Most frequently reported adverse reactions were fever, rhinitis, cough, exanthema, conjunctivitis, diarrhea and arthralgias. There were no severe reactions or hospitalizations. The aerosol group showed significantly lower frequency of fever, rhinitis, cough, conjunctivitis and arthralgias than the subcutaneous group (Table 2).

#### 3.2. Rubella immunogenicity

Both the aerosol and the subcutaneous group showed an increase in post-vaccination seropositivity rates. The aerosol vaccine produced similar seroconversion and post-vaccination seropositivity rates as the subcutaneous vaccine. When geometric means of titters of antibodies were examined, both groups increased post-vaccination levels of antibodies and the increase was similar for both groups.

### Table 1

**Characteristics of study participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MR subcutaneous, ( n = 307 )</th>
<th>MR aerosol, ( n = 255 )</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (x, S.D.)</td>
<td>6.52 (0.59)</td>
<td>6.45 (0.51)</td>
<td>0.1</td>
</tr>
<tr>
<td>Girls (n, %)</td>
<td>149 (48.5)</td>
<td>125 (49.0)</td>
<td>0.9</td>
</tr>
<tr>
<td>Nutritional status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malnourished (n, %)</td>
<td>17 (5.6)</td>
<td>20 (7.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Well nourished (n, %)</td>
<td>270 (89.4)</td>
<td>221 (86.7)</td>
<td></td>
</tr>
<tr>
<td>Obese (n, %)</td>
<td>15 (5.0)</td>
<td>14 (5.5)</td>
<td></td>
</tr>
<tr>
<td>Previous measles vaccination (n, %)</td>
<td>307 (100)</td>
<td>255 (100)</td>
<td></td>
</tr>
<tr>
<td>Previous rubella vaccination</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Rubella pre-vaccine antibodies (ELISA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n, %)</td>
<td>138 (44.9)</td>
<td>119 (46.7)</td>
<td>0.5</td>
</tr>
<tr>
<td>Geometric mean (IU/ml)</td>
<td>16</td>
<td>17</td>
<td>0.2</td>
</tr>
<tr>
<td>Measles pre-vaccine antibodies (ELISA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n, %)</td>
<td>182 (59.3)</td>
<td>169 (66.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Negative (n, %)</td>
<td>86 (28.0)</td>
<td>48 (18.8)</td>
<td></td>
</tr>
<tr>
<td>Indeterminate (n, %)</td>
<td>39 (12.7)</td>
<td>38 (14.9)</td>
<td></td>
</tr>
<tr>
<td>Measles pre-vaccine antibodies (PN)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n, %)</td>
<td>30 (96.7)</td>
<td>24 (85.7)</td>
<td>0.1</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>609.88</td>
<td>708.91</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\( ^a \chi^2\) and Wilcoxon rank sum.

\( ^b \geq 15\) IU/ml.

\( ^c \geq 120\) IU/l.

\( ^d \) For 59 children (subcutaneous 31, aerosol 29).
This was so, both when post-vaccination titers for all participants were considered, as well as when post-vaccination titers were considered only for those who seroconverted (Table 3). Seroconversion rates decreased with increasing levels of antibodies present before vaccination. No difference for geometric means of antibody titers between groups was observed at each level of pre-vaccination antibody titer (Table 4). By multivariate analysis, no variables other than baseline seronegativity ($P < 0.001$) were significant for rubella response.

### 3.3. Measles immunogenicity

Both groups increased post-vaccination measles seropositivity rates. The aerosol vaccine produced significantly higher seroconversion and post-vaccination seropositivity rates than the subcutaneous vaccine. This difference was observed when individuals with negative and indeterminate titers at pre-vaccination were considered separately or jointly. In the subsample with PN results, all vaccinated subjects became seropositive after vaccination in both groups. Seroconversion rates were significantly higher in the aerosol group. When geometric means were compared, the aerosol group had higher post-vaccination titers, both when all vaccinated subjects were considered, and when only seroconverters were analyzed (Table 3), while controlling for baseline antibody titer. Multivariate analysis revealed that the aerosol-administered vaccine produced significantly more seroconversions (OR = 14.49, 95% CI 3.95–53.13, $P = 0.0001$). Inclusion of age, gender and nutritional status did not modify this model.

Four-fold or greater increases in NT titers were noted in one or more students in six of the seven schools given aerosol, but in only one of the 10 schools injected vaccine ($P = 0.004$, Fisher’s) was given. Further, the average frequency of such responses by school was also significantly higher ($P = 0.025$) for schools where aerosol was given (51.4%), than in those schools where injected vaccine was given (10%).

### 4. Discussion

This study has demonstrated that the administration of combined MR aerosol vaccines, as a booster dose, is as
antibodies. The high immunogenicity of strain RA27/3 has
addition to the expected superior mucosal immunity, our
ation that mucosal (intranasal) immunization with RA27/3
published. Evidence has been presented supporting the no-
subcutaneous route for rubella immunization have been
then, no clinical trials comparing the aerosol route with
was successfully achieved by Ganguly in 1973 [6,7]. Since
ation is also extended to women of childbearing ages [26].
by an increase in congenital rubella syndrome, unless vacci-
for Disease Control and Prevention, who processed sam-
lyophilized and liquid) and the SmithKline Beecham Lab-
and V accines Institute, Berna, Switzerland (EZ/RA27/3
Acknowledgements
Study vaccines were kindly provided by the Swiss Serum
Study provided data indicating that aerosol route can
be used to administer combination of antigens. For those
wishing to conduct vaccinations campaigns with these vac-
cines, the aerosol route is equally immunogenic in eliciting
rubella antibodies, superior for measles seroconversion, and
likely to elicit superior mucosal immunity for both.

References
[1] Sabin AB. My last will and testament on rapid elimination and
ultimate global eradication of poliomyelitis and measles. Pediatrics
[2] Couts PF, Clements CJ, Bennett JV. Alternative routes of measles


[16] Albrecht P. Measles plaque reduction neutralization test in 24-well tissue culture trays, standard operating procedure developed and established by the Food and Drug Administration. Food and Drug Administration, 1980.


