Clinical and Serologic Evaluation of Measles, Mumps, and Rubella (HPV-77:DE-5 and RA 27/3) Virus Vaccines, Singly and in Combination

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ABSTRACT. A double-blind, placebo-controlled comparison of single component and combination measles, mumps, and rubella (HPV-77:DE-5 and RA 27/3) virus vaccines involving 502 young children was conducted. The rubella antibody response was similar with RA 27/3 rubella and measles-mumps-rubella (RA 27/3) vaccines, but was diminished with the combination vaccine that incorporated HPV-77:DE-5 rubella. There was no evidence of enhanced clinical reactivity with either of the measles-mumps-rubella vaccines. *Pediatrics* 68:18–22, 1981; *measles-mumps-rubella vaccine, measles vaccine, mumps vaccine, rubella vaccine, viral interference, viral vaccines.*

When a new component is introduced into a live attenuated viral vaccine combination, it must be demonstrated that the antibody response is not diminished and that clinical reactions are not enhanced. Wistar RA 27/3 rubella virus vaccine is felt to have greater immunologic potency than HPV-77:DE-5 rubella virus vaccine. This study was conducted to evaluate RA 27/3 rubella vaccine as a replacement for HPV-77:DE-5 rubella vaccine in combination measles-mumps-rubella virus vaccine. Furthermore, the study was specifically designed to explore the feasibility of conducting a double-blind, placebo-controlled vaccine trial as part of ongoing health care in multiple pediatric clinics and private offices.

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MATERIALS AND METHODS

Vaccines and Placebo

We tested the following six live attenuated viral vaccines: more attenuated Enders' (Moraten) measles; Jeryl Lynn mumps; HPV-77:DE-5 rubella; measles-mumps-rubella, with HPV-77:DE-5 as the rubella component; Wistar RA 27/3 rubella; and measles-mumps-rubella, with Wistar RA 27/3 as the rubella component. The RA 27/3 strain of rubella virus was isolated from an aborted rubella-infected human fetus and attenuated by 29 passages in WI-38 human diploid fibroblast tissue culture. The two RA 27/3 rubella virus vaccines, single component and combination, were tested for potency and safety by the Merck Institute for Therapeutic Research. The four other vaccines were commercial products of Merck Sharp & Dohme. The placebo consisted of vaccine diluent. All vials were relabeled with a code number and contents were unknown to those who administered and those who received the vaccine. We entered three times as many vials of each of the two combination vaccines as each of the single component vaccines and placebo.

Study Population

The children were recruited into the study when they came to their clinic or physician's office for routine measles-mumps-rubella virus vaccination. They were 15 months to 4 years of age, healthy, and had negative disease and vaccination histories for measles, mumps, and rubella. Children with temperature ≥100.5 F were excluded, but children with afebrile upper respiratory infection were not.

Informed Consent

Parents read a detailed description of the background, procedures, benefits, and hazards of the study that had been approved by the University of Nebraska Institutional Review Board for the Protection of Human Subjects, the University of Nebraska Medical Center Human Investigation and Ethics Committee, and the United States Air Force Surgeon General's Clinical Investigation Committee. They were also encouraged to ask questions before signing the consent form.

Plan of Study

Each participating clinic or office had a supply of randomly selected coded vaccine or placebo vials. A vial was picked, diluent added, and the contents administered subcutaneously. The parent was given a 42-day record that listed a variety of specific local and systemic symptoms and also contained space for open-ended comments. The parent also received a thermometer, along with instruction in its use, and was urged to take and record the temperature if the child appeared ill in any way. The clinic or office scheduled a six weeks return appointment. At that time, the symptom record was reviewed and additional details were elicited, if necessary. At the conclusion of the return visit, a sealed box bearing the child's code number was taken from the refrigerator and opened. If the child had initially received a single component vaccine or placebo, the box contained vaccine incorporating the two or three viral antigens that the child had not received. If the child had initially received one of the two combination vaccines, the box contained no vaccine. In this way, we gave the children all three viral antigens without repetition of any antigens and still maintained the double-blind nature of the study. Blood samples for serologic testing were drawn just before, and six weeks after, vaccination.

Conduct of Study

We contacted all 15 private pediatric practices in the metropolitan Omaha area about the study. Five private practices, ranging in size from solo to six-person, agreed to participate. In addition, a university medical center pediatric clinic, the immunization clinics of two metropolitan Omaha counties, and the immunization clinic of a nearby air force base participated in the study. At the air force base clinic, a research nurse (M.B. or J.B.), assisted by a phlebotomist, carried out the study procedure following initial recruitment by clinic personnel. In the other settings, medical, nursing and laboratory personnel conducted the study without outside assistance.

Participating clinics and private offices were paid a stipend of $25 for each child who completed the study to compensate them for the extra time and effort expended and for the additional patient visit required. Patients were not charged for vaccine or for the six weeks return visit.

The study extended from May 1977 to April 1979.

Serologic Testing

Antibody titers were measured at the Merck Institute for Therapeutic Research under code. Standard hemagglutination inhibition (HI) techniques for measles and rubella were employed,[1,2] using serial twofold serum dilutions starting at 1:5 for measles and 1:8 for rubella and expressing results as the reciprocal of the highest serum dilution that caused complete inhibition of hemagglutination. A newly developed indirect immunofluorescent assay was used to measure mumps antibody concentrations (FIAX, International Diagnostic Technology, Santa Clara, CA 95050; M. M. Hitchings, unpublished data, 1978). The degree of fluorescence measured by this assay yielded continuous values according to the amount of antibody in the serum sample. These values were correlated with the results of the standard mumps neutralizing antibody test, so that an immunofluorescent value of 2 corresponded to the presence of neutralizing antibody at a serum dilution of 1:2.

Clinical Reactions

Symptoms and signs were analyzed for their occurrence within the following intervals: days 0 to 4, 5 to 12, 13 to 18, 19 to 28, 29 to 42, and 0 to 42. Maximum temperatures were grouped as follows: <99 F, 99 to 100.9 F, 101 to 102.9 F, and 103 to 104.9 F. Rashes were classified as either a specific dermatologic entity such as diaper rash, pityriasis rosea, or varicella, or as a localized (one area of the body) or generalized (two or more areas of the body) nonspecific erythematous maculopapular rash.

Statistical Methods

Serologic results were compared by analysis of variance and clinical reactions by Fisher's exact test.

RESULTS

Serologic

There were 502 children in the study. Two sera, prevaccination and six weeks postvaccination, were obtained from 308 (61%) children and were used to

analyze serologic responses. An additional 135 children (27%) had *either* a pre- or a postvaccination serum sample taken, but not both. Serum was unobtainable at both visits from only 59 (12%) children.

Prior to vaccination, 96%, 82%, and 94% of the children were seronegative for measles, mumps, and rubella, respectively. As shown in Table 1, the mumps antibody titer of one child changed from <2 to 6 during the six weeks following placebo injection. The proportions of children who developed detectable antibody for each of the three antigens were similar, whether the antigen was given alone or as part of a combination product. Among those who become seropositive, the measles geometric mean titers (GMT) for the measles-containing vaccines were similar. Mumps induced a higher GMT as part of measles-mumps-rubella (RA 27/3) vaccine than as part of measles-mumps-rubella (HPV-77:DE-5) vaccine ($P < .05$), although neither was significantly different from the GMT of mumps vaccine alone. Rubella antibody levels were similar when RA 27/3 rubella was given as a single component vaccine or in a combination vaccine, but levels were significantly lower ($P < .05$) with measles-mumps-rubella (HPV-77:DE-5) vaccine than with single component HPV-77:DE-5 rubella vaccine. The rubella GMTs induced by HPV-77:DE-5 rubella vaccine were as high as those of RA 27/3 rubella and measles-mumps-rubella (RA 27/3) vaccines.

Clinical

Local and systemic symptoms and signs during the 42-day study period and during time intervals within this period, analyzed for just those children with documented seroconversion or for the total group, were similar with each vaccine; they were no greater than experienced by placebo recipients. Selected reaction rates for the total study group are shown in Table 2.

None of the children who received either of the two single component rubella vaccines had arthritis or arthralgia. Only one child in each of the two combination vaccine groups had mild arthralgia and, moreover, the early onset in both instances suggested that it was unrelated to vaccine: the hips of one child seemed sore when his diaper was changed on days 5, 6, and 15 after receiving measles-mumps-rubella (HPV-77:DE-5) vaccine and the shoulders and knees of another child seemed stiff on days 1 and 2 following administration of measles-mumps-rubella (RA 27/3) vaccine. Neither fever nor rash was more frequent or severe on days 5 to 12 in children given any of the three measles-containing vaccines than in placebo recipients. Two children had rashes that may have been allergic in nature and are not included among the nonspecific generalized erythematous maculopapular rashes in Table 2. However, concurrent illness, rather than vaccine, may have been responsible: one child had nonpruritic wheals on his face on days 1 to 10 after administration of RA 27/3 rubella vaccine in association with fever, lymphadenopathy, and a variety of respiratory and gastrointestinal symptoms; another child had a rash diagnosed by a physician as erythema multiforme along with fever, otitis media, and diaper rash on days 8 to 12 following administration of measles-mumps-rubella (HPV-77:DE-5) vaccine.

Conduct of Study

Of the 502 study volunteers, 298 (59%) were seen at the air force base immunization clinic in which the research nurse worked three mornings per week. The university medical center clinic and the county immunization clinics recruited 129 (26%) children. The five private pediatric practices together entered 75 (15%) children in the study, with the number from each practice ranging from two to 49. In these various settings, blood drawing success rates

TABLE 1. Development of Antibody in Initially Seronegative Children Six Weeks Following Administration of Placebo or Vaccine

Placebo/Vaccine	No. Paired Sera	Measles		Mumps		Rubella	
		%*	GMT†	%	GMT	%	GMT
Placebo	23	0	...	5	6	0	...
Measles	23	100	82				
Mumps	33			92	19		
HPV-77:DE-5 rubella	20					95	296‡
Measles-mumps-rubella (HPV-77:DE-5)	85	99	77	89	15‡	99	144‡
RA 27/3 rubella	33					100	306
Measles-mumps-rubella (RA 27/3)	91	96	89	90	31‡	100	301

* Percent of those who developed detectable antibody.
† Geometric mean titer of those who became seropositive.
‡ $P < .05$ for mumps GMTs with measles-mumps-rubella (HPV-77:DE-5) vs measles-mumps-rubella (RA 27/3) and for rubella GMTs with HPV-77:DE-5 rubella vs measles-mumps-rubella (HPV-77:DE-5).

TABLE 2. Proportion (%) of Children Who Experienced Symptoms and Signs During the Six Weeks Following Administration of Placebo or Vaccine

Placebo/Vaccine	No.	Local Reaction*	Fever (F)		Respiratory Symptoms	Rash†	Lymphadenopathy	Sore Eyes	Joint Symptoms
			101–102.9	103–104.9					
Placebo	42	7	24	0	74	9	0	9	0
Measles	43	2	28	5	79	12	2	14	0
Mumps	41	14	15	7	63	2	5	19	0
HPV-77:DE-5 rubella	47	6	13	6	66	13	4	17	0
Measles-mumps-rubella (HPV-77:DE-5)	142	5	22	8	68	17	4	17	0.7
RA 27/3 rubella	46	4	24	4	67	11	4	17	0
Measles-mumps-rubella (RA 27/3)	141	8	25	11	72	20	8	16	0.7

* Pain, redness, or swelling at the injection site on days 0 to 4.
† Nonspecific erythematous maculopapular rash involving two or more areas of the body.

and parental cooperation in recording clinical observations and returning for the six weeks visits were similar.

DISCUSSION

Wistar RA 27/3 rubella vaccine has potential immunologic advantages over HPV-77:DE-5 and Cendehill rubella vaccines. In addition to stimulating HI antibody, RA 27/3 rubella vaccine regularly induced serum complement fixation, neutralization, and iota-precipitin antibodies,[3-9] evoked secretory IgA antibody,[3,10-12] and gave a high degree of protection against reinfection with rubella virus challenge.[3,13,14] With these characteristics, RA 27/3 rubella vaccine closely resembled natural rubella infection. However, like the other two vaccines, it was noncontagious and had low reaction rates in children.[3,4,15,16] Furthermore, because it was grown in human tissue culture, RA 27/3 rubella vaccine obviated the potential problem of allergic reaction to nonhuman protein.

This study confirmed that HPV-77:DE-5 and RA 27/3 rubella vaccines show a comparably high degree of effectiveness in eliciting serum HI antibody and that both have very low reactivity rates in young children. This study also established for the first time that measles and mumps vaccines neither diminished antibody response nor enhanced clinical reactivity when combined with RA 27/3 rubella vaccine.

However, we unexpectedly found that rubella HI antibody titers were significantly lower when HPV-77:DE-5 rubella vaccine was administered in combination with measles and mumps vaccines than when it was given alone. Previous field trials of measles-mumps-rubella (HPV-77:DE-5) vaccine,[17,18] as well as mumps-rubella (HPV-77:DE-5) vaccine[19] and measles-rubella (HPV-77:DE-5) vaccine,[20,21] had shown no such interference, either in terms of seroconversion rates or GMTs. These previous field trials, in contrast to the current study, involved comparisons of the rubella-containing combination vaccines with single component rubella vaccine that was not administered concurrently. This disparate finding needs to be further investigated, in view of the large number of children who have received measles-mumps-rubella (HPV-77:DE-5) vaccine since 1969.

There are precedents for immunologic interference when two or more live virus vaccines are given simultaneously and at the same site. When vaccinia virus and 17D yellow fever virus were combined, the dermal response to vaccinia was normal, but the number of yellow fever seroconversions was reduced.[22,23] There was no suppression of the yellow fever antibody response when vaccinia and yellow fever were given simultaneously, but at separate sites.[22,24,25] Meyer et al.[26] found that combining measles or measles and yellow fever with smallpox vaccine reduced the level of smallpox antibody, without reducing the number of seroconverions or the dermal responses. The yellow fever seroconversion rate was also reduced somewhat.

The presumed mechanism for these observations is local induction by one virus of a substance, such as interferon, which inhibits replication of another virus, thus reducing or eliminating the amount of antigenic stimulation. Local interferon has been detected in vaccinia virus vaccination crusts,[27] but local interferon stimulated by measles virus vaccine has not been studied. Whatever the mechanism, it appears from this study that HPV-77:DE-5 rubella virus is more susceptible to local inhibition than RA 27/3 rubella virus.

We were unable to detect any significant differences in the rates of fever and rash when children who received more attenuated Enders' (Moraten) measles vaccine, either singly or in one of the two combinations, were compared with placebo recipients. This observation stands in contrast to several previous controlled trials with the same measles

vaccine that found fever (≥101 F) rates of 6% to 20% and rash rates of 8% to 18%.[28-30] The difference relative to fever may have been due to a specific difference in study design. We asked the parent to take the temperature if the child appeared ill in any way; previous studies involved routine daily temperatures during the critical period five to 12 days postvaccination. It has been noted that children with fever from measles vaccine were often asymptomatic, and fever without accompanying illness would not have been detected in our study. We have no explanation for the differences relative to rash.

Ethical considerations have made increasingly untenable the conduct of vaccine studies in circumscribed groups of retarded, poor, or otherwise handicapped children. Therefore, new vaccines must be tested in open populations that are not subject to unusual medical or social pressures. Our experience indicates that a large double-blind vaccine trial can be carried out as part of routine health care in a variety of clinic and office settings, with the active support of interested medical, nursing, and laboratory personnel.

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