Clinical evaluation of a new measles–mumps–rubella combined live virus vaccine in the Dominican Republic *

N. JOEL EHRENKRANZ,[1] ARNOLDO K. VENTURA,[2] EDWARD M. MEDLER,[3] JOSEPH E. JACKSON,[4] & MICHAEL T. KENNY [5]

Over 900 children were enrolled in a double-blind placebo-controlled clinical study of measles (Schwarz strain), mumps (Jeryl Lynn strain), and rubella (Cendehill strain) trivalent vaccine. The trivalent vaccine caused about the same degree of reactivity as is generally associated with the Schwarz strain measles vaccine. Paired sera from triple-susceptible vaccinees had seroconversion rates of 99% for measles, 94% for mumps, and 93% for rubella. The results of this study show that this trivalent vaccine is as well tolerated and as effective as its component vaccines.

The growing list of virus diseases becoming amenable to control by vaccination necessitates improvements in the system for delivery of this type of preventive medical care. The efficiency of immunization programmes, both private and public, can be greatly increased by the use of polyvalent vaccines provided that the individual components were all intended for the same populations. The polyvalent combinations should be no more reactive and no less effective than the components used singly. For live, attenuated virus vaccines, both the practical and technical requirements have been met by a variety of double and triple combinations (1, 2) each prepared for use in certain countries or populations.

A double-blind, placebo-controlled study was conducted to evaluate clinically a new measles-mumps-rubella trivalent live virus vaccine. Consultation with health authorities in the Dominican Republic revealed that country to be an appropriate site for a project of this type. In intercity and rural populations the use of measles vaccine had been so recently introduced that it had not yet made an impact on the age-specific susceptibility rates. In these same populations, mumps and rubella vaccines had not yet come into use.

MATERIALS AND METHODS

Vaccines

The vaccine was a combination of live attenuated measles virus vaccine, Schwarz strain (3, 4), live mumps virus vaccine, Jeryl Lynn strain (5–7), and live rubella virus vaccine, Cendehill strain (8, 9). Three lots of this trivalent vaccine were used in these studies. Each lot was made from separate monovalent components and contained a minimum of 1 000 $TCID_{50}$ (50% tissue culture infective doses) of measles and rubella vaccine viruses and 5 000 $TCID_{50}$ of mumps vaccine virus per dose. The trivalent vaccine was dispensed into single dose vials, lyophilized, and stored at 5°C until use. Single-dose vials were reconstituted with sterile diluent from prefilled syringes just prior to subcutaneous inoculation. The vaccine and placebo were produced at the Biological Laboratories of the Dow Chemical Co. The placebo was prepared in a similar manner but without the addition of any virus, and was indistinguishable from the vaccine whether lyophilized or reconstituted.

Serology

Antibodies to measles and rubella viruses were determined by the hemagglutination-inhibition (HI)

* From Cedars of Lebanon Hospital, Miami, FL 33125, USA, and the Human Health Research Laboratories, Dow Chemical Co., Indianapolis, IN 46268, USA. Requests for reprints should be addressed to Dr Ehrenkranz.

[1] Chief of Medicine, Cedars of Lebanon Hospital.

[2] Department of Medicine, Cedars of Lebanon Hospital.

[3] Associate Clinical Investigator, Dow Chemical Co.

[4] Director, Biological Clinical Research, Dow Chemical Co.

[5] Senior Research Immunologist, Dow Chemical Co.

method (10, 11). Antibodies to mumps virus were determined by the serum neutralization (SN) method. Serum for HI testing was pretreated with kaolin (for measles) or with manganese chloride and heparin (for rubella) and then adsorbed with the appropriate test erythrocytes (i.e., African green monkey erythrocytes for measles, newly-hatched chick erythrocytes for rubella). The HI assays were done in microtitre plates, in which 0.025-ml volumes of serial 2-fold dilutions of treated serum were tested against 4 hemagglutination (HA) units of Norrby-type measles HA antigen, or against 4 HA units of commercial rubella HA antigen. The highest dilution of serum completely inhibiting hemagglutination was taken as the antibody endpoint. Although this microtitre system yields considerably lower titres than the tube hemagglutination technique, its convenience for large-scale work outweighed this disadvantage. Pairs of serum samples that failed to show obvious seroconversion in the microtitre system were retested by the conventional tube technique (12).

Mumps antibody titres were determined by the VERO cell microtitre serum neutralization (SN) technique (13). If postinoculation antibody was not detected by this technique, paired serum samples were retested undiluted and at 1 : 2 dilution by a plaque reduction technique in VERO cells (14).

Children were considered susceptible to a disease if antibody to that disease was not detectable (measles HI titre $<1:2$, mumps SN titre $<1:2$, rubella HI titre $<1:8$) in the preinoculation serum. Seroconversion after inoculation was determined by the appearance of antibody in the postinoculation serum sample of a previously susceptible child. Serum samples were always tested as pairs. All serologic assays were double blind. Results were then entered into a computer for automated decoding and calculation and tabulation of seroconversion rates and geometric mean titres.

Study design

A randomized double-blind design was used to distribute the study population among the 3 lots of vaccine and 1 lot of placebo in the ratio of 1 placebo subject to every 5 vaccinees. Each vial of vaccine or placebo was labelled with a unique serial number that became the subject number of the child inoculated from that vial. A blood sample was obtained prior to vaccination. Clinical observations for possible vaccine reactions and intercurrent illnesses were made approximately 3 times per child during the study period from about day 7 to day 21. The results of these observations were decoded and tabulated by computer. A second blood sample was taken 8 weeks after vaccination. The children who had originally received the placebo were then vaccinated.

Study population

The children admitted to this study were residents of the Herrera District of Santo Domingo, Dominican Republic. Requirements for inclusion in the study were: (a) informed consent of the parents; (b) absence of any history of natural measles, mumps, or rubella, or of immunization against these diseases; and (c) absence of all the usual medical contraindications to immunization with live, attenuated virus vaccine.

RESULTS

Clinical findings

A total of 926 children participated in the study. Their age distribution and age-specific susceptibility rates are shown in Table 1. The sharp drop in triple susceptibility rate in those aged 2 and 3 years is due mainly to natural measles infection. The apparently higher triple susceptibility rate in those 5 and 6 years of age is an artifact probably caused by the small number of that age sampled. Other serologic surveys of children in and around Santo Domingo revealed no such unevenness in the decline of triple susceptibility rate with age (15). There were no reports of any reactions at the injection site, and no immediate or early adverse systemic effects of the injection.

Table 1. Age distribution and age-specific triple susceptibility rates in the study population

Age in years	Number of subjects	Triple susceptible (%)
1	181	50.3
2	179	26.8
3	209	14.3
4	247	7.3
5	94	10.6
6	4	50.0
7	1	0.0
unknown	11	9.1
total	926	21.6

Table 2. Distribution of maximum axillary temperature readings from the 7th to the 21st day after vaccination

Temperature range (°C)	Triple-susceptible vaccinees (all lots)	Placebo subjects
36.7	6 (3.7) [a]	6 (4.7) [a]
36.7–37.2	122 (74.8)	96 (75.6)
37.3–37.7	27 (16.6)	22 (17.3)
37.8–38.3	3 (1.8)	2 (1.6)
38.4–38.8	1 (0.6)	1 (0.8)
38.9–39.4	2 (1.2)	0
39.5–40.0	2 (1.2)	0
total	163	127

[a] Figures in parentheses are percentages.

The distribution of axillary temperature readings from the 7th to the 21st day after vaccination are shown in Table 2. The vaccinees showed slightly higher frequencies than those given placebos at the upper limits of the temperature range, but these differences in distribution were not statistically significant (Fisher's Exact Probability: $P = 0.22$ for temperatures >37.8°C and $P = 0.18$ for temperatures >38.3°C).

The frequencies of clinical findings other than fever during the 2nd and 3rd weeks after vaccination are shown in Table 3. Rash was slightly more frequent in triple-susceptible vaccinees (6.7%) than in susceptible placebo subjects (4.7%). Although this slight excess of rash in vaccinees is similar to that which may occur with measles vaccine used singly, the difference between vaccinees and placebo controls in this study was not statistically significant ($\chi^2 = 0.53$, $P > 0.1$). None of the other clinical findings showed any statistically significant difference between triple-susceptible vaccinees and placebo controls. The most frequent finding was rhinitis, which was reported more frequently in placebo subjects (43.3%) than in triple-susceptible vaccinees (39.9%). Symptoms that were specifically looked for but not found in this study population included bronchitis, parotitis, orchitis, and paraesthesia.

Table 3. The frequencies of positive clinical findings from the 7th to the 21st day after vaccination

	Triple-susceptible vaccinees (all lots)	Susceptible placebo subjects
rash	11 (6.7) [a]	6 (4.7) [a]
lymphadenopathy	1 (0.6)	2 (1.6)
conjunctivitis	7 (4.3)	5 (3.9)
otitis media	4 (2.5)	1 (0.8)
coryza	3 (1.0)	1 (0.8)
rhinitis	65 (39.9)	55 (43.3)
pharyngitis	2 (1.2)	2 (1.6)
cough	1 (0.6)	0
headache	1 (0.6)	1 (0.8)

[a] Figures in parentheses are percentages.

Joint pain was reported for only one child, a 4-year-old triple-susceptible male vaccinee. It was described as minimal, and occurred on the 14th day after vaccination at which time the child also had rhinitis, pharyngitis, and an axillary temperature of 37.8°C. This child was free of symptoms on subsequent observations.

Miscellaneous clinical findings unrelated to vaccination, but characteristic of this tropical intercity population, included a wide variety of enteric, respiratory, and dermatologic symptoms. Such findings were as prevalent in placebo subjects as in triple-susceptible vaccinees.

Serologic results

Postvaccination serum samples were obtained from 439 children, of whom 84 were triple-susceptible. The serologic results obtained with these triple-susceptible subjects are shown in Table 4. In 72 susceptible vaccinees the seroconversion rates were 99% for measles, 94% for mumps, and 93% for rubella. The geometric mean antibody titres were 35.6 for measles, 3.9 for mumps, and 34.4

Table 4. Immune responses of 72 triple-susceptible children inoculated with the trivalent vaccine and of 12 triple-susceptible children inoculated with placebo

Seroconversion to:	triple-susceptible vaccinees			Placebo subjects
	No.	%	GMT [a]	
measles	71	99	35.6	0
mumps	68	94	3.9	0
rubella	67	93	34.4	0

[a] Geometric mean titre.

for rubella. These values are no different from those usually found following the use of the same virus strains as monovalent vaccines. There were no seroconversions among the 12 triple-susceptible placebo subjects, or among 47 others with various single and double susceptibilities.

DISCUSSION

The occurrence of transient fever within the range 38.9°C–40.0°C in 4 (2.3%) of the triple-susceptible vaccinees and none of the placebo controls is consistent with that which would be expected following administration of the Schwarz strain measles vaccine alone (4). This, together with the lack of any statistically significant difference between the distributions of temperature readings in triple-susceptible vaccinees and placebo subjects, indicates that combining the vaccines did not enhance their known minor febrile effects. The same may be said of rash, the frequency of which was only 2% higher in triple-susceptible vaccinees than in placebo subjects, and was well within the range generally reported for the Schwarz strain measles vaccine (4, 16). Comparison of the frequencies of these and other symptoms in triple-susceptible trivalent vaccinees and placebo subjects indicates that there is no enhancement of side effects when the three vaccines are combined to make a trivalent vaccine.

The evidence also indicates that the 3 vaccines are as effective when given together as when used individually. Since the seroconversion rates and geometric mean titres induced by the trivalent vaccine are quantitatively equivalent to those generally induced by the component vaccines used singly, it is to be expected that the qualitative aspects of the immune response, particularly its long duration, will also be the same for the trivalent vaccine.

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RÉSUMÉ

ÉVALUATION CLINIQUE EN RÉPUBLIQUE DOMINICAINE D'UN NOUVEAU VACCIN VIVANT MIXTE ANTIROUGEOLEUX, ANTIOURLIEN ET ANTIRUBÉOLEUX

Un vaccin trivalent antirougeoleux (souche Schwartz), antiourlien (souche Jeryl Lynn) et antirubéoleux (souche Cendehill) a fait l'objet, en République Dominicaine, d'une étude clinique portant sur 926 enfants et visant à déterminer le taux de réactions cliniques ainsi que l'efficacité de ce vaccin comme agent immunisant. Trois lots du vaccin en question ont été comparés à un placebo, la méthode du double insu étant employée de telle façon qu'un enfant sur six, désigné au hasard, reçoive le placebo. Un échantillon de sang a été prélevé sur chaque enfant avant la vaccination. Du septième ou vingt et unième jour inclus suivant la vaccination, on a procédé, toujours selon la méthode du double insu, à environ trois examens cliniques de chaque enfant pour détecter les réactions postvaccinales et les maladies intercurrentes possibles. Un dernier échantillon de sang a été prélevé huit semaines après la vaccination et les enfants qui avaient reçu le placebo ont alors été vaccinés.

Des données cliniques ont été obtenues chez les 290 enfants suivis, dont 163 vaccinés sensibles aux trois virus en cause et 127 sujets ayant reçu le placebo. On a observé de la fièvre un peu plus fréquemment parmi les vaccinés que parmi les autres, mais la différence n'est pas statistiquement significative. Une éruption s'est produite chez 6,7% des vaccinés sensibles aux trois virus et chez 4,7% des sujets ayant reçu le placebo. La proportion légèrement plus élevée d'éruptions dans le groupe des vaccinés sensibles correspond à ce qu'on aurait pu escompter avec un vaccin antirougeoleux monovalent, la différence n'étant pas statistiquement significative. La conjonctivite a également été plus fréquente parmi les vaccinés sensibles, mais la différence est inférieure à 1%. Un seul enfant, vacciné sensible, a souffert de douleurs articulaires; elles ont été minimes et de courte durée.

L'analyse des échantillons de sang prélevés avant et après la vaccination sur 84 enfants sensibles aux trois

virus, dont 72 sujets vaccinés et 12 sujets ayant reçu le placebo, a donné les résultats suivants: parmi les vaccinés, acquisition d'une immunité à la rougeole chez 99%, la moyenne géométrique des titres étant de 35,6, d'une immunité aux oreillons chez 94% (moyenne: 3,9), et d'une immunité à la rubéole chez 93% (moyenne: 34,4).

Ces données cliniques et sérologiques indiquent que les trois vaccins considérés présentent la même sécurité d'emploi et la même efficacité lorsqu'ils sont administrés ensemble que lorsqu'ils sont utilisés séparément.

REFERENCES

1. MEYER, H. M. ET AL. *In:* First International Conference on Vaccines against Viral and Rickettsial Diseases of Man, Washington, DC, November, 1966. Washington, DC, Pan American Health Organization, 1967, pp. 336-342.
2. REY, M. *In:* Proceedings of the International Conference on the Application of Vaccines against Viral, Rickettsial, and Bacterial Diseases of Man, Washington, DC, December, 1970. Washington, DC, Pan American Health Organization, 1971, pp. 407-412.
3. SCHWARZ, A. J. F. *Annales de pédiatrie*, **202**: 241-252 (1964).
4. SCHWARZ, A. J. F. ET AL. *Journal of the American Medical Association*, **199**: 26-30 (1967).
5. WEIBEL, R. E. ET AL. *New England journal of medicine*, **276**: 245-251 (1967).
6. HILLEMAN, M. R. ET AL. *New England journal of medicine*, **276**: 252-258 (1967).
7. HILLEMAN, M. R. ET AL. *New England journal of medicine*, **278**: 227-232 (1968).
8. DUPAN, R. M. ET AL. *American journal of diseases of children*, **115**: 658-662 (1968).
9. PRINZIE, A. ET AL. *American journal of diseases of children*, **118**: 172-177 (1969).
10. LINNEMANN, C. C. JR ET AL. *American journal of epidemiology*, **95**: 238-246 (1972).
11. COOPER, L. Z. ET AL. *Journal of the American Medical Association*, **207**: 89-93 (1969).
12. ROSEN, L. *Virology*, **13**: 139-141 (1961).
13. KENNY, M. T. ET AL. *Applied microbiology*, **20**: 371-373 (1970).
14. KENNY, M. T. ET AL. *Journal of biological standardization*, **3**: 291-306 (1975).
15. KENNY, M. T. ET AL. *American journal of epidemiology*, **103** (1976) (in press).
16. ANDELMAN, M. B. ET AL. *American journal of public health*, **56**: 1891-1897 (1966).