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STUDIES ON LIVE RUBELLA VACCINE. V. QUANTITATIVE ASPECTS OF INTERFERENCE BETWEEN RUBELLA, MEASLES AND MUMPS VIRUSES IN THEIR TRIVALENT VACCINE

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SUMMARY Rubella vaccine combined with measles and mumps vaccines was administered in a single injection to children of 1 to 5 years of age. All three vaccines were serologically effective, and the clinical reactions caused by measles vaccine were considerably alleviated, when 6×10^3 PFU of rubella and 10^4 TCD_{50} per dose of mumps vaccines were combined with 5×10^4 TCD_{50} of measles vaccine. When a larger amount of mumps vaccine (3×10^5 TCD_{50}/dose) was used, it caused interference with the rubella and measles viruses, i.e., the antibody response to rubella virus became poor and the incidence of clinical reactions to measles virus decreased. On the other hand, when 5×10^5 TCD_{50}/dose of measles vaccine was combined with 10^4 TCD_{50}/dose of mumps vaccine, the clinical reactions to measles virus were decreased but were almost the same as those induced by this vaccine alone.

INTRODUCTION

The effectiveness and safety of trivalent live measles, mumps and rubella virus vaccine have been well documented (Buynak et al., 1969; Smorodintsev et al., 1970; Stokes et al., 1971; Krugman et al., 1971) as well as those of divalent rubella and mumps vaccine (Weibel et al., 1971) and measles and rubella vaccine (Landrigan et al., 1973). The observed antibody response and clinical reactions were similar to those following vaccinations with each vaccine alone. On the other hand Buynak et al. (1969) reported that the choice of a particular strain and the level of its attenuation had considerable affects on serological and clinical responses, although these authors did

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not investigate these factors in detail. Moreover the quantitative relationship between the three vaccines has not been elucidated fully.

We examined the effects of various proportions of measles, mumps and rubella vaccines to determine the quantities in trivalent vaccine required to immunize children effectively. Results using a single injection of the trivalent vaccine are reported in this communication.

MATERIALS AND METHODS

1. *Vaccines*

Live rubella vaccine was prepared by freezing and thawing from quail embryo fibroblast cells infected with the Matsuura strain (Okuno et al., 1968; Minekawa et al., 1968, 1973). Lot #7318 (E65QEF-15) (passaged 65 times in the amniotic cavity of chick embryos and 15 times in quail embryo fibroblast cells) was used throughout this work.

Live measles vaccine was prepared from CEF (chick embryo fibroblast)cells infected with the Tanabe strain of measles virus (Ueda et al., 1970a, 1970b) and was designated as CAM-A4.

Mumps vaccine was prepared from the amniotic fluid of chick embryos infected with the Urabe strain Am9, as reported elsewhere (Yamanishi et al., 1970, 1973).

Divalent live vaccine, MM01, is a combination of measles CAM-A4 and mumps Am9 vaccines.

All these vaccines were lyophylized in ampoules and dissolved in 0.5 ml of 0.01 M PBS(−) (phosphate-buffered saline without divalent cations) before use.

For passage of the vaccine strains in embryonated chicken eggs, RIF (resistance-inducing factor) (Rubin, 1960)-free eggs were used, as reported elsewhere (Minekawa et al., 1973).

2. *Vaccinees*

Healthy children aged 1 to 5 years in an open community were inoculated subcutaneously in the upper part of the arm with a single dose of 0.5 ml of the vaccine.

3. *Serological assay*

Blood specimens were taken from the vaccinees 4 or 5 weeks after immunization. Antibody titers of rubella were measured by the hemagglutination-inhibition (HI) test (Suzuki et al., 1973) modified from the original method by Stewart et al. (1967), and those of measles or mumps by the neutralization (NT) test in microtiter plates, as reported elsewhere (Ueda et al., 1972; Yamanishi et al., 1973).

4. *Survey for clinical reactions*

All the vaccinees were observed clinically and their body temperature was measured daily for 4 weeks by their parents. Vaccinees with a body temperature of more than 37.5 C on days 5 to 15 after vaccination were regarded as showing a febrile reaction.

RESULTS

1. *Effect of dilution of rubella vaccine in trivalent live vaccine*

Rubella lot# 7318 vaccine evoked a favorable antibody response without any particular clinical reaction when administered alone. However, when the vaccine was combined with MM01 vaccine after 10-fold dilution with 0.01 M PBS(−), the seroconversion rate and geometric mean antibody titer (GMT) to rubella virus became lower than those with undiluted vaccine (Table 1). This suggests that MM01 vaccine might interfere with rubella virus, reducing the antibody response to it. Clinically, combined use of the three vaccines reduced the incidence of febrile reactions (mainly to measles vaccine because rubella and mumps vaccines do not cause febrile reactions) from about 50% with monovalent measles vaccine (Ueda et al., 1970b) to 25.0%, or to 16.7% after 10-fold dilution of the MM01 vaccine. This suggests that one (or both) of the other viruses interfered with measles virus. This is one of the merits of the combined form of vaccine. Dilution of the rubella vaccine had no influence on the rate of febrile reactions, so rubella virus probably does not interfere with measles virus. Although the incidence of febrile reactions decreased as mentioned above, there was little difference in the incubation period, ca. 6 days, max fever, ca. 38.8 C, duration of fever, ca. 2 days, or incidence of the rash, ca. 10%, from those observed after monovalent vaccine administration (Ueda et al., 1970b).

TABLE 1. *Effects of dilution of rubella vaccine on serological responses and clinical reactions to trivalent live vaccines*

Vaccine	Rubella 7318 (6×10^3 PFU/dose) MM01 (measles 5×10^5 TCD_{50}/dose, mumps 10^5 TCD_{50}/does)		Rubella 7318 (1:10) (6×10^2 PFU/dose) MM01 (measles 5×10^5 TCD_{50}/dose, mump 10^5 TCD_{50}/does)		Rubella 7318 (1:10) alone (6×10^2 PFU/dose)	
Antigen	Seroconversion (No. positive[a]/ No. tested)	GMT[b]	Seroconversion (No. positive/ No. tested)	GMT	Seroconversion (No. positive/ No. tested)	GMT
Rubella	15/16 (93.7%)	5.8	14/18 (77.8%)	4.1	5/5 (100%)	5.2
Measles	16/16 (100%)	4.9	18/18 (100%)	4.6	—	
Mumps	16/16 (100%)	3.3	18/18 (100%)	2.6	—	
Clinical reaction						
Fever	4/16[c] (25.0%)		3/18 (16.7%)		0/5 (0%)	
Incubation	6.3 days		6.0 days		—	
Max fever	38.6 C		39.0 C		—	
Duration	1.8 days		2.0 days		—	
Rash	1/16 (6.3%)		2/18 (11.1%)		0/5 (0%)	

[a] Antibody titers of 1:8 or more by the HI test for rubella virus and of 1:2 or more by the NT test for measles and mumps viruses were evaluated as positive.

[b] Geometric mean antibody titer, expressed as $\log_2$.

[c] Number of vaccinees who had a febrile reaction of higher than 37.5 C per total number examined on days 5 to 15 after vaccination.

2. *Effects of dilution of MM01 vaccine*

The effects of dilution of the measles and mumps vaccines on the serological and clinical effects of rubella vaccine were tested. As shown in Table 2, the seroconversion rate to rubella virus was better with diluted MM01 vaccine than with undilued vaccine and the GMT was rather higher than that following monovalent vaccine administration.

Clinically, when the measles vaccine was diluted 10-fold to 5×10^4 TCD_{50}/dose, the rate of the febrile reaction, the incidence of a rash and the duration of fever decreased considerably though the interfering effect of mumps virus should decrease equally on dilution. Judging from these results, this latter combination seems better as a trivalent vaccine from the view points of antibody responses and clinical reactions.

3. *Effects of dilution of mumps vaccine on rubella or measles vaccine*

The interfering effects of mumps virus were confirmed by testing trivalent vaccines containing various dilutions of mumps vaccine. A high titer of 3×10^5 TCD_{50}/dose of mumps vaccine diminished the seroconversion rate and GMT of rubella vaccine as well as the febrile reaction of measles vaccine (Table 3). However, decrease in the inoculum of mumps vaccine improved the seroconversion rate and GMT of rubella vaccine and 100-fold diluted mumps vaccine (10^4 TCD_{50}/dose) did not interfere much with rubella or measles vaccine. Consequently clinical reactions caused by measles vaccine, such as the febrile reaction, increased to almost the same level as those obtained with monovalent CAM-A4 vaccine.

This phenomenon was shown in another way.

TABLE 2. *Effects of dilution of measles and mumps vaccines on serological responses and clinical reactions to trivalent live vaccines*

Vaccine	Rubella 7318 (6×10^3 PFU/dose) MM01 (measles 5×10^5 TCD_{50}/dose, mumps 10^5 TCD_{50}/dose)		Rebella 7318 (6×10^3 PEU/dose) MM01 (1 : 10) (measles 5×10^4 TCD_{50}/dose, mumps 10^4 TCD_{50}/dose)		Rubella 7318 alone (6×10^3 PFU/dose)	
Antigen	Seroconversion (No. positive[a]/ No. tested)	GMT[b]	Seroconversion (No. positive/ No. tested)	GMT	Seroconversion (No. positive/ No. tested)	GMT
Rubella	35/44 (79.5%)	5.9	8/9 (88.9%)	6.6	25/25 (100%)	5.6
Measles	44/44 (100%)	5.9	9/9 (100%)	5.6	—	
Mumps	43/44 (97.7%)	3.1	9/9 (100%)	3.2	—	
Clinical reaction						
Fever	11/44[c] (25.0%)		1/9 (11.1%)		0/25 (0%)	
Incubation	6.1 days		6.0 days		—	
Max fever	38.4 C		38.0 C		—	
Duration	1.9 days		1.0 day		—	
Rash	2/44 (4.5%)		0/9 (0%)		0/25 (0%)	

a, *b* and *c* as for Table 1.

TABLE 3. *Effects of dilution of mumps vaccine on serological responses and clinical reactions to trivalent live vaccines*

Vaccine	Rubella 7318 (6×10^3 PFU/dose) Measles CAM-A4 (3×10^5 TCD_{50}/dose) Mumps Am9 (1 : 3) (3×10^5 TCD_{50}/dose)		Rubella 7318 (6×10^3 PFU/dose) Measles CAM-A4 (3×10^5 TCD_{50}/dose) Mumps Am9 (1 : 10) (10^5 TCD_{50}/dose)		Rubella 7318 (6×10^3 PFU/dose) Measles CAM-A4 (3×10^5 TCD_{50}/dose) Mumps Am9 (1 : 100) (10^4 TCD_{50}/dose)	
Antigen	Seroconversion (No. positive[a]/ No. tested)	GMT[b]	Seroconversion (No. positive/ No. tested)	GMT	Seroconversion (No. positive/ No. tested)	GMT
Rebella	3/17 (17.7%)	3.0	20/26 (76.9%)	5.0	13/14 (92.9%)	4.6
Measles	17/17 (100%)	5.5	26/26 (100%)	5.5	14/14 (100%)	6.1
Mumps	17/17 (100%)	2.9	25/26 (96.2%)	3.0	13/14 (92.9%)	2.9
Clinical reaction						
Fever	1/17[c] (5.9%)		3/26 (11.5%)		7/14 (50.0%)	
Incubation	7.0 days		6.7 days		5.9 days	
Max fever	38.6 C		38.8 C		38.9 C	
Duration	2.5 days		2.2 days		1.9 days	
Rash	1/17 (5.9%)		2/26 (7.7%)		2/14 (14.3%)	

a, *b* and *c* as for Table 1.

Namely, as shown in Fig. 1, the seroconversion rate of rubella vaccine and the incidence of febrile reactions to measles vaccine seemed to be proportional to the dilution of mumps vaccine, i.e., with increased dilution of mumps virus, the antibody response to rubella vaccine and the incidence of febrile reactions increased.

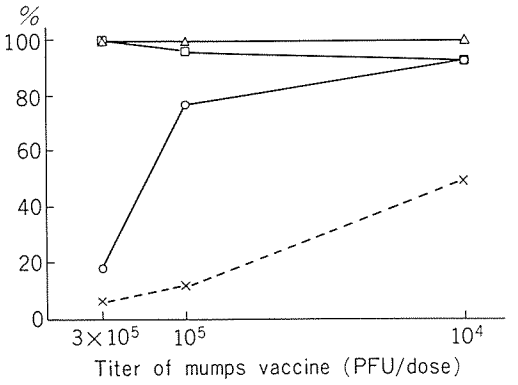

FIGURE 1. *Relationship between mumps vaccine titer and seroconversion rate or febrile reaction after inoculation with trivalent live vaccines.* ○——○, *seroconversion rate of rubella vaccine;* △——△, *seroconversion rate of measles vaccine;* □——□, *seroconversion rate of mumps vaccine;* ×——×, *incidence of febrile reaction.*

Serologically, 10^4 TCD_{50}/dose of mumps vaccine in the combined vaccine appeared best. Clinically, 3×10^5 TCD_{50}/dose of measles vaccine appeared too high for combined use with 10^4 TCD_{50}/dose of mumps vaccine, causing 50% febrile reactions, but as shown in Table 2, 5×10^4 TCD_{50}/dose of measles vaccine caused only 11.1% febrile reactions when combined with this amount of mumps virus. Thus the most suitable combination in the trivalent live vaccine appears to be 10^4 TCD_{50}, 5×10^4 TCD_{50} and 6×10^3 PFU per dose of mumps, measles and rubella vaccines, respectively.

DISCUSSION

Monovalent rubella vaccine is useful for protection of young adult women of child-bearing age against a possible rubella epidemic in the near future. However it is more convenient to vaccinate young children or infants with polyvalent vaccine against epidemics in the far future when they reach child-bearing age, since this is simpler to administer, it is cheaper and above all it minimizes the number of vaccinations necessary. However, combination with other vaccines might decrease the seroconversion rate or GMT of rubella vaccine and further investigations, such as those reported here, are necessary on this possibility.

Tests of combinations of the three vaccines at various dilutions revealed that using 10^4 TCD_{50}, around 10^4 TCD_{50} and more than 10^3 PFU per dose of mumps, measles and rubella vaccines, respectively, in the trivalent vaccine was satisfactory. This vaccine showed one of the merits of combined vaccine, namely decreased clinical reactions to measles virus, and did not show the demerit of decrease in the seroconversion rate and GMT of rubella vaccine.

Interference in trivalent live vaccine may be caused not only quantitatively, as shown here, but also qualitatively in proportion to the degree of attenuation of the vaccines combined. In the latter case more attenuation of the virus which causes interference was found to increase production of interferon (Meyer et al., 1966), although it is unknown if this sort of interference is mediated entirely by interferon. On the other hand, more attenuation of the virus suffering interference would result in slower growth and increased susceptibility to interference. For example mumps vaccine caused great interference with an extremely attenuated CAM-EX measles vaccine (Ueda et al., 1972), judging from serological results (Ueda et al., unpublished data). The finding described in this report that in the trivalent vaccine the antibody response to rubella virus was inhibited but that to measles virus was not, suggests that the grades of attenuation of the rubella and measles viruses used were different. Using more attenuated rubella virus in this

vaccine, growth would be inhibited and so not reach a high enough level to induce an antibody response. With slightly less attenuated measles virus growth would also be inhibited, but only to a level which was not enough to evoke clinical reactions but was enough to induce an antibody response. Namely there might be two quantitative levels in replication of a virus, the lower one inducing an antibody response only and the higher one also causing clinical reactions. In another words, attenuation of a virus might be partly explained by the level of multiplication which the virus could reach. Further experiments using various vaccines at different levels of attenuation are necessary to clarify these points. Follow-up studies are also required on the duration of the antibody and its effectiveness in protection against epidemics.

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REFERENCES

Buynak, E. B., R. E. Weibel, J. E. Whitman, Jr., J. Stokes, Jr., and M. R. Hilleman. 1969. Combined live measles, mumps, and rubella virus vaccines. JAMA 207: 2259–2262.

Krugman, S., G. Muriel, and V. J. Fontana. 1971. Combined live measles, mumps, rubella vaccine. Am. J. Dis. Child. 121: 380–381.

Landrigan, P. J., K. B. Murphy, H. M. Meyer, Jr., P. D. Parkman, D. L. Eddins, and J. J. Witte. 1973. Combined measles-rubella vaccines. Virus dose and serological response. Am. J. Dis. Child. 125: 65–67.

Meyer, H. M., Jr., P. D. Parkman, and T. C. Panos. 1966. Attenuated rubella virus. II. Production of an experimental live virus vaccine and clinical trial. N. Engl. J. Med. 275: 575–580.

Minekawa, Y., N. Suzuki, M. Takahashi, and Y. Okuno. 1968. Studies on live rubella vaccine. II. Field trials with two experimental vaccine strains. Biken J. 11: 243–250.

Minekawa, Y., N. Suzuki, J. Osame, S. Ueda, K. Yamanishi, M. Takahashi, Y. Okuno, T. Kan, N. Onishi, and H. Toyama. 1973. Studies on live rubella vaccine. IV. Comparative field trials with attenuated vaccines at different passage levels in Japanese quail embryo fibroblast cells. Biken J. 16: 155–160.

Okuno, Y., Y. Minekawa, N. Suzuki, and M. Takahashi. 1968. Studies on live rubella vaccine. I. Propagation of rubella virus in developing chick embryos and its laboratory characterization. Biken J. 11: 235–241.

Rubin, H. 1960. A virus in chick embryos which induces resistance in vitro to infection with Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. 46: 1105–1119.

Smorodintsev, A. A., M. N. Nasibov, and N. V. Jakovleva. 1970. Experience with live rubella virus vaccine combined with live vaccines against measles and mumps. Bull. WHO 42: 283–289.

Stewart, G. L., P. D. Parkman, H. E. Hopps, R. D. Douglas, J. P. Hamilton, and H. M. Meyer, Jr. 1967. Rubella-virus hemagglutination-inhibition test. N. Engl. J. Med. 276: 554–557.

Stokes, J., Jr., R. E. Weibel, V. M. Villarejos, J. A. Arguedas, E. B. Buynak, and M. R. Hilleman. 1971. Trivalent combined measles-mumps-rubella vaccine. Findings in clinical-laboratory studies. JAMA 218: 57–61.

Suzuki, N., Y. Minekawa, J. Osame, S. Ueda, T. Ogino, K. Yamanishi, M. Takahashi, Y. Okuno, T. Kan, N. Onishi, and K. Koyama. 1973. Studies on live rubella vaccine. III. Field trials with vaccines attenuated by serial passages in primary pig kidney cells. Biken J. 16: 149–154.

Ueda, S., M. Takahashi, Y. Minekawa, T. Ogino, N. Suzuki, K. Yamanishi, K. Baba, Y. Okuno, T. Konobe, T. Sasada, and K. Takaku. 1970a. Studies on further attenuated live measles vaccine. I. Adaptation of measles virus to the chorioallantoic membrane of chick embryo and clinical tests on the strain. Biken J. 13: 111–116.

Ueda, S., M. Takahashi, T. Kurimura, T. Ogino, N. Suzuki, K. Yamanishi, K. Baba, Y. Okuno, T. Konobe, T. Sasada, K. Onishi, and K. Takaku. 1970b. Studies on further attenuated live measles vaccine. IV. Clinical and serological evaluation of a clone of CAM-CEF measles vaccine virus. Biken J. 13: 169–174.

Ueda, S., M. Takahashi, T. Kurimura, Y. Mine-

kawa, N. Suzuki, K. Yamanishi, K. Baba, Y. Okuno, T. Sasada, K. Onishi, T. Konobe, K. Takaku, Y. Yamada, Y. Tanami, F. Kusano, Y. Hayakawa, and T. Kurose. 1972. Development of extremely attenuated live measles virus vaccine (CAM-EX). Biken J. 15: 173–177.

Weibel, R. E., J. Stokes, Jr., V. M. Villarejos, J. A. Arguedas, E. B. Buynak, and M. R. Hilleman. 1971. Combined live rubella-mumps virus vaccine. Findings in clinical-laboratory studies. JAMA 216: 983–986.

Yamanishi, K., M. Takahashi, T. Kurimura, S. Ueda, Y. Minekawa, T. Ogino, N. Suzuki, K. Baba, and Y. Okuno. 1970. Studies on live attenuated mumps virus vaccine. III. Evaluation of newly developed live mumps virus vaccine. Biken J. 13: 157–161.

Yamanishi, K., M. Takahashi, S. Ueda, Y. Minekawa, T. Ogino, N. Suzuki, Y. Okuno, and T. Kan. 1973. Studies on live mumps virus vaccine. V. Development of a new mumps vaccine "Am 9" by plaque cloning. Biken J. 16: 161–166.