JOURNAL OF CLINICAL MICROBIOLOGY, June 1977, p. 584-587 Vol. 5, No. 6
Copyright © 1977 American Society for Microbiology Printed in U.S.A.

Clinical Trial with "R-75" Strain Live, Attenuated, Serum Inhibitor-Resistant Intranasal Influenza B Vaccine

MARY J. SPENCER,* JAMES D. CHERRY, AND KEITH R. POWELL

Department of Pediatrics, Division of Infectious Diseases, University of California at Los Angeles School of Medicine, Los Angeles, California 90024

Received for publication 6 January 1977

The "R-75" strain live, attenuated, serum inhibitor-resistant influenza B vaccine was administered intranasally by drops in two doses 14 days apart to 21 volunteers. Each vaccinee was paired with a close associate (roommate or workmate) who similarly received two doses of a placebo solution. Although about 50% of both vaccine and placebo recipients complained of symptoms after dosage, the severity of symptoms was greater in vaccine recipients. Fourfold serum hemagglutination-inhibiting antibody titer rises occurred in 38% of vaccine recipients, and four vaccinees had fourfold titer rises of nasal hemagglutination-inhibiting antibody. Vaccine virus was isolated from three asymptomatic vaccine recipients. There was no virological or serological evidence of the vaccine virus spreading to placebo recipients.

Although influenza A viruses are responsible for most major influenza epidemics and all pandemics, considerable morbidity and mortality can be attributed to influenza B viral infections (8). In recent years, inactivated influenza A vaccines have proven successful in the prevention of disease caused by influenza A viral strains (7). Modern influenza B vaccines are probably also useful in influenza B prophylaxis, but only vague evidence of efficacy is available. A major drawback of all inactivated viral vaccines is the requirement for frequent booster doses.

In the past decade, new methods of attenuating influenza viruses have been developed that have facilitated production of candidate live viral vaccines (6). We have recently observed promising results in trials with a live, attenuated, candidate influenza A vaccine (10). Encouraged by these findings, we undertook the presently described study with "R-75" strain influenza B vaccine.

MATERIALS AND METHODS

Vaccine and placebo. R-75 strain vaccine is a recombinant of influenza B/Hong Kong/5/72 and the serum inhibitor-resistant influenza strain B/Russia/69. The recombinant is not inhibited by horse and guinea pig sera and has the antigenic characteristics of B/Hong Kong/5/72. It was produced by Recherche et Industrie Therapeutiques, Brussels, Belgium, and supplied by Smith, Kline and French Laboratories, Philadelphia, Pa., in multiple-dose vials of lyophilized material ($10/^{7.5}$ mean egg infective doses/dose). The vaccine was reconstituted immediately prior to use with 2.5 ml of a 5% sucrose solution and administered by dropper (5 drops/nostril) to a supine subject with the head extended for 1 min.

The placebo, supplied in single-dose vials, was allantoic fluid reconstituted just prior to use with 0.5 ml of a 5% sucrose solution and administered by dropper (5 drops/nostril) in a manner identical to that for R-75 strain vaccine.

Study population. Twenty-one pairs of UCLA students and employees who either lived together or worked in close proximity to each other were selected for participation in the study. Informed consent was obtained from all participants. Volunteers were of both sexes, and their ages ranged from 24 to 50 years old.

Procedure of study. After a medical history was compiled and a physical examination performed, blood was obtained for a complete blood count and glucose, creatinine, urea nitrogen, glutamic oxaloacetic transaminase, alkaline phosphatase, and bilirubin values; a urine sample was also procured for routine studies and microscopic examination. By single blind control, one of each volunteer pair received two doses 14 days apart of R-75 strain vaccine intranasally. In a similar manner, the other participant in the pair received the placebo solution. Blood for serological study was obtained on days 0, 14, and 30. Nasal swabs for attempted viral isolation were collected on days 0, 1, 2, and 7 after each dosage and placed in 2 ml of Hanks balanced salt solution containing 0.5% bovine serum albumin and standard antibiotics. Specimens for viral isolation were inoculated into tissue culture immediately after collection. Specimens for nasal antibody studies were obtained on days 0, 14, and 30 by a nasal wash with 10 ml of phosphate-buffered saline solution followed by blowing.

At the time of immunization, volunteers were asked to report immediate taste sensations. In addi-

tion, study participants were asked to record and grade, on a scale of 0 to 3 (0, none; 1, mild; 2, moderate; 3, severe), their specific clinical symptoms for each of 7 days after every immunization.

Virus isolation techniques. Each day 0 specimen was inoculated into two tubes each (0.1 ml/tube) of rhesus monkey kidney (MK) and WI38 tissue cultures. Subsequent specimens (0.1 ml) were inoculated into MK tissue cultures and frequently also into chicken embryo trachea organ cultures. Maintenance fluid was Eagle basal medium with standard antibiotics. Cultures were incubated at 35°C on roller drums (15 revolutions per hour) and examined three times weekly; the medium was changed every 3 to 4 days. Hemadsorption with 0.4% guinea pig erythrocytes was performed on all MK tissue cultures without cytopathic effect on day 14. All MK tissue cultures and chicken embryo trachea organ cultures were blind-passed once in MK tissue cultures and examined for hemadsorption on day 14, if a cytopathic effect had not been observed.

Serological techniques. Influenza B hemagglutination-inhibiting (HAI) antibody studies were performed by standard microtiter methods (9). All sera were initially treated with receptor-destroying enzyme, and all assays were done on paired specimens.

Nasal washings were homogenized, concentrated with Sephadex G-200, and examined for immunoglobulin A, total protein, and influenza B HAI and neutralizing antibody. Samples for the study of nasal HAI antibody were treated with receptor-destroying enzyme. Total nasal protein was measured by optical density at 280 nm against a standard curve obtained with crystalline bovine serum albumin. Immunoglobulins were determined by radial immunodiffusion with a 7S immunoglobulin A serum standard. Resultant values were adjusted to 11S secretory immunoglobulin A levels by multiplying times a factor of three (10).

To determine nasal neutralizing antibody titers, the Sephadex G-200 concentrates were diluted 1:1 with Eagle basal medium containing 0.5% gelatin and antibiotics, heat-inactivated at 56°C for 30 min, serially diluted and mixed with an equal volume of a virus mixture containing 32 mean tissue culture infective doses ($TCID_{50}$) of virus per 0.1 ml, and incubated at room temperature for 1 h. A portion (0.2 ml) of each serum-virus mixture was added to each of two MK tissue cultures. Cultures were observed daily for cytopathic effect and examined for hemadsorption on day 6.

RESULTS

Clinical findings. Immediately after the first and second intranasal administrations 62% and 57%, respectively, of vaccine recipients and 48% of placebo recipients noted an unusual taste sensation. Since there was little difference between the taste reported by vaccine or placebo recipients, it is most likely that the taste sensation was due to the allantoic fluid, which was in the placebo as well as the vaccine, rather than the vaccine virus itself.

Frequency and severity of clinical symptoms during the two 7-day observation periods after vaccine or placebo administration are presented in Table 1. Fifty-seven percent of vaccine recipients and 52% of placebo recipients had one or more symptoms after the initial dose; an equal number of vaccine and placebo recipients had one or more symptoms after the second dose.

Whereas the overall frequency as noted above did not differ to any degree between vaccine recipients and those receiving the placebo, the symptom index was considerably greater in vaccine recipients; this difference was largely due to severe and multiple influenza-like complaints in three vaccinees. Rhinitis, cough, pharyngitis, headache, malaise, and myalgia were all more prominent in vaccine recipients than in placebo recipients.

A physical examination and routine laboratory studies were performed on each individual at the initial visit and on day 30. No study participant had any physical or laboratory abnormality that could be associated with vaccination.

Vaccine virus isolation studies. To determine the sensitivity of our viral isolation systems, R-75 strain vaccine was titrated in MK tissue cultures and chicken trachea organ cultures. Reconstituted vaccine with an initial titer of $10^{7.2}$ mean egg infective doses 0.1 ml had a titer of 10^{7} $TCID_{50}$/0.1 ml and $10^{6.5}$ $TCID_{50}$/0.1 ml in MK tissue cultures and chicken trachea organ cultures, respectively. Vaccine virus was isolated from three vaccine recipients (Table 2). From one subject, virus was recovered on day 1 after both the initial and second immunizations. Of the other two isolates, one occurred on day 2 after the first immunization and the other resulted from a day 2 specimen after the second dose. All isolates were in MK tissue cultures and were only noted after blind passage. Participants from whom virus was isolated had no

TABLE 1. *Frequency and severity of clinical symptoms in R-75 strain vaccine and placebo recipients*

Recipients	Percent with one or more symptoms	Symptom index[a]
R-75 strain vaccine		
Dose 1	57	7.3
Dose 2	43	4.7
Placebo		
Dose 1	52	2.9
Dose 2	43	2.0

[a] Symptom index = total symptom grade for 7 days/number receiving vaccine or placebo.

TABLE 2. *Summary of 13 R-75 strain vaccinees with evidence of influenza B viral infection*

Evidence of infection with R-75 vaccine	Study no.	Serum HAI antibody on day			Nasal HAI antibody on day			Nasal neutralizing antibody on day			Virus shed	Symptoms
		0	14	30	0	14	30	0	14	30		
Virus shed	4	10	10	10	ND[a]	<2	<2	ND	<2	<2	Vaccine 2, day 2	No
	26	10	10	20	<2	<2	<2	ND	<2	<2	Vaccine 1, day 1 Vaccine 2, day 1	No
Virus shed and fourfold serum HAI antibody titer rise	1	<5	10	20	ND	<2	2	ND	<2	3	Vaccine 1, day 2	No
	10	5	20	20	<2	<2	<2	<2	<2	<2	No	Mild
	21	20	80	80	<2	<2	<2	<2	2	2	No	Mild
Fourfold rise in serum HAI antibody titer	22	10	40	40	<2	<2	<2	<2	<2	3	No	No
	25	10	10	80	<2	<2	ND	<2	<2	ND	No	Mild
	33	5	10	20	<2	<2	<2	<2	<2	<2	No	No
	40	10	40	10	<2	<2	<2	<2	<2	<2	No	Mild
Fourfold serum and nasal antibody titer rises	20	20	80	80	<2	64	<2	<2	ND	8	No	Mild
	8	10	10	10	ND	<2	4	ND	2	4	No	No
Fourfold nasal antibody rise	14	40	40	20	<2	<2	8	<2	<2	16	No	No
	35	10	10	10	<2	8	<2	2	2	<2	No	No

[a] ND, Not done.

associated clinical findings. Vaccine virus was not isolated from any placebo recipients.

Serology. The serum geometric mean influenza B antibody titer of R-75 strain vaccinees rose from initial values of 9 to 14 on day 14 and 18 on day 30. No change in the influenza B HAI antibody titer in placebo recipients occurred. Thirty-eight percent of R-75 vaccinees had fourfold serum influenza B HAI antibody titer rises; no placebo recipient had a similar fourfold titer rise. Three of the five vaccinees with initial HAI titers ≤ 5 had fourfold antibody titer rises, whereas only 25% of those vaccinees with initial titers ≥ 10 had similar titer rises.

Nasal-wash specimens from days 0, 14, and 30 were available from 13 vaccine and 15 placebo recipients. Three vaccinees (23%) had fourfold titer rises in nasal HAI antibody, and two of these three also had a fourfold neutralizing antibody titer response. In two instances, the nasal antibody response was apparently transitory, since it was noted in day 14 but not in day 30 specimens.

Only one subject with a nasal antibody response had a concomitant fourfold serum antibody titer rise, and none of the three vaccinees from whom virus was isolated had a nasal antibody response, although one of these three volunteers did have a fourfold rise in serum HAI antibody titer.

When viral isolation results and serum and nasal antibody studies are considered together, 13 of the 21 (62%) vaccinees had evidence of infection with the vaccine virus (Table 2). There was no serological or virological evidence of a spread of vaccine viral infection to placebo recipients. It is also to be noted that clinical symptomatology could not be correlated with either virological or serological evidence of vaccine viral infection. In fact, the symptom index (symptom index = total symptom grade for both 7-day observation periods/number of vaccinees with or without infection) in vaccinees with serological or virological evidence of infection was less (index, 6.8) than that in vaccinees without evidence of infection (index, 24.5).

DISCUSSION

The availability of an effective, live, attenuated influenza B vaccine would be a welcome addition to influenza immunoprophylaxis. Since a major antigenic shift apparently does not occur with influenza B viruses and since minor antigenic changes are less frequent than with influenza A viruses, it would appear that successful immunoprophylaxis could be obtained with attenuated influenza B viruses. An obvious advantage of a live intranasally administered vaccine would be the elimination of yearly intramuscular booster doses. Presumably, booster doses with a live vaccine would be unnecessary. At times of significant antigenic change, a new attenuated virus would need to be developed and utilized.

Although the majority of R-75 strain vaccine recipients noted a distinct taste immediately after immunization, this effect was not considered objectionable. Most volunteers stated they preferred intranasal to parenteral vaccine administration. The limited size of the present study makes a comparative statistical evaluation of symptomatology difficult. Approximately 50% of both vaccine and placebo recipients had one or more complaints during the 7-day observation periods after dosages; this finding would suggest that complaints were not vaccine related. However, by the symptom index, it would appear that the vaccine was indeed responsible for some illness. Previous trials by other investigators with candidate, attenuated, influenza B vaccines have also revealed some reactogenicity (1-5). This contrasts with our findings from an inhibitor-resistant, live, attenuated influenza A vaccine (10). Beare et al. (2, 4) found that symptomatology in vaccine recipients correlated directly with antibody response. In contrast, the three vaccinees in this study with the most marked symptomatology had neither virological nor serological evidence of infection.

Vaccine virus was recovered from three vaccine recipients, indicating an isolation rate similar to that noted by others (2, 4, 5). Since the vaccine is prepared in eggs, it is probable that egg inoculation would have been a more sensitive indicator of infection. Interestingly, chicken embryo trachea organ cultures were less sensitive than MK tissue cultures. Although viral multiplication is an obvious necessity for successful immunization with a live virus, the amount of virus shed is so small that any spread from vaccinees to others appears unlikely. Even during the close contact between vaccine and placebo recipients in this study, no transmission occurred. This lack of human spread could be important, as Beare et al. (1) noted increased virulence after human passage of an attenuated influenza B strain.

Serum antibody responses after immunization with R-75 strain vaccine were modest. However, three of five vaccinees with initial HAI antibody titers ≥ 5 had fourfold HAI antibody titer rises after immunization. It would appear that pre-immunization antibody protected against vaccine viral infection. In a study comparing live and killed influenza B virus vaccines, Beare et al. (2) noted that 71% of those given killed vaccine developed a serum antibody response, whereas only 51% of live virus vaccinees had a similar antibody response. However, when challenged intranasally with live virulent influenza B virus, 15% of those initially receiving inactivated vaccine were infected, whereas only 2% of the live virus vaccine recipients were infected.

When compared with the results of our similar trials with "Alice" strain live, attenuated influenza A vaccine, it would appear that the R-75 strain is not a very promising vaccine candidate (10). However, the most important test of vaccine efficacy is the demonstration of protection from natural challenge. It is quite possible that the relatively poor serum and nasal antibody responses after R-75 strain immunization were not due to factors of the vaccine per se, but to the fact that the volunteer population was relatively immune to influenza B strains with the B/Hong Kong/72 antigenic makeup. A more meaningful evaluation could perhaps have been performed if vaccination had been restricted to subjects known to be seronegative for B/Hong Kong/72 antibody.

ACKNOWLEDGMENTS

This study was supported by a grant from Smith, Kline and French Laboratories, Philadelphia, Pa.

LITERATURE CITED

1. Beare, A. S., M. L. Bynoe, and D. A. J. Tyrrell. 1968. Investigation into the attenuation of influenza viruses by serial passage. Br. Med. J. 4:482-484.
2. Beare, A. S., D. Hobson, S. E. Reed, and D. A. J. Tyrrell. 1968. A comparison of live and killed influenza-virus vaccine. Lancet ii:418-420.
3. Beare, A. S., H. F. Maassab, D. A. J. Tyrrell, A. N. Slepuskin, and T. S. Hall. 1971. A comparative study of attenuated influenza viruses. Bull. W. H. O. 44:593-598.
4. Beare, A. S., D. A. J. Tyrrell, D. Hobson, C. H. L. Howells, M. S. Pereira, T. M. Pollock, and L. E. Tyler. 1969. Live influenza B vaccine in volunteers. J. Hyg. 67:1-11.
5. Downie, J. D., and C. H. Stuart-Harris. 1970. The production of neutralizing activity in serum and nasal secretion following immunization with influenza B virus. J. Hyg. 68:233-244.
6. Kilbourne, E. D. 1975. New approaches to the prevention of influenza. Acta Med. Scand. 5576:45-48.
7. Kilbourne, E. D., R. M. Chanock, R. W. Choppin, F. M. Davenport, J. P. Fox, M. B. Gregg, G. G. Jackson, and P. D. Parkman. 1974. Influenza vaccines - summary of influenza workshop V. J. Infect. Dis. 129:750-771.
8. Knight, V., and J. A. Kasel. 1973. Influenza viruses. *In* V. Knight (ed.), Viral and mycoplasmal infections of the respiratory tract. Lea and Febiger, Philadelphia.
9. Robinson, R. Q., and W. R. Dowdle. 1969. Influenza viruses, p. 87-123. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections, 4th ed. American Public Health Association, Inc., New York.
10. Spencer, M. J., J. D. Cherry, K. R. Powell, C. B. Sumaya, and A. J. Garakian. 1975. Clinical trials with Alice strain, live attenuated, serum inhibitor-resistant intranasal influenza A vaccine. J. Infect. Dis. 132:415-420.