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A clinical trial of WRL 105 strain live attenuated influenza vaccine comparing four methods of intranasal vaccination

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SUMMARY

A single intranasal dose of $10^{7.0}$ EID50 recombinant WRL 105 strain live attenuated influenza vaccine was administered intranasally to 193 volunteers either as nose drops or by one of three spray devices which produced sprays of differing physical characteristics. In volunteers with homologous haemagglutinating inhibiting antibody titres of $\leqslant 20$ before vaccination, seroconversion rates varied widely from 80% following the administration of drops to 71%, 57% and 28% with the three spray devices.

In the week following vaccination 16 (22%) of 74 volunteers who were found to show a fourfold or greater antibody response took analgesics to control symptoms in comparison with 4 (7%) of 58 volunteers who exhibited no serological response to vaccination ($P < 0{\cdot}05$). However, neither the occurrence of upper respiratory nor systemic symptoms were significantly different in these two groups and the degree of attenuation of the recombinant WRL 105 strain appears to be acceptable for future use.

INTRODUCTION

Many clinical trials of various strains of live influenza vaccine have been carried out in the United Kingdom during the last ten years (McDonald, Zuckerman, Beare & Tyrrell, 1962; Beare, Hobson, Reed & Tyrrel, 1968; Beare *et al.* 1971; Freestone *et al.* 1972; Beare, Habershon, Tyrrell & Hall, 1973). Most trials have not involved the vaccination of large numbers of subjects and as until recently no other live vaccine was licensed for intranasal use, devices to facilitate intranasal vaccination have not been developed. This report describes the results of a study undertaken to compare four methods of intranasal vaccination and to carry out a first open community assessment of recombinant WRL 105 strain live attenuated influenza vaccine. WRL 105 strain is a recombinant of A/Okuda/57 (280th egg passage level) and A/Finland/4/74 strains prepared by methods described by McCahon & Schild (1972). The A/Okuda/57 strain grows well in

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Table 1. *Reasons why volunteers were not vaccinated*

Total number of volunteers not vaccinated:	27 of 220 (12·3 %)
Coryza at the time of vaccination	6
Influenza at the time of vaccination	1
Past history of Asthma	4
Past history of Asthma and Bronchitis	1
Chronic Bronchitis	3
Recent attack of Bronchitis & Coryza	1
Recurrent or Chronic Sinusitis	6
Recurrent Sinusitis and Bronchitis	1
Chronic Catarrh and occasional Bronchitis	1
Quiescent Chronic Suppurative Otitis Media activated by upper respiratory tract infections	1
Vaso-Motor Rhinitis (recent polypectomy)	1
Not recorded	1

eggs and was selected as the attenuated parent for recombination since its history is limited to passage in eggs and it was shown to be attenuated in clinical trials in adults and in children when its antigenic characteristics were relevant (Okuno & Nakamura, 1966). The antigenic characteristics of the A/Finland/4/74 strain are similar to those of A/Port Chalmers/1/73. However, A/Finland/4/74 is a more recently isolated strain and shows some antigenic drift towards the A/Scotland/840/74 strain which has been isolated with increasing frequency in the United Kingdom and in other countries during the winter of 1974/75. In initial small scale trials recombinant WRL 105 influenza vaccine has been found to be immunogenic, non-transmissible, of acceptable reactivity (Moffat, M. A. J., Stealey, V. M., Freestone, D. S. & Macdonald, A. Personal communication) and to confer protection against A/Scotland/840/74 (Morris, Freestone, Stealey & Oliver, 1975).

Freeze-dried vaccine prepared from the recombinant WRL 105 strain was produced in accordance with the draft recommendations of the Medical Research Council.

METHODS

During January 1975, 220 employees of British Leyland Limited volunteered to take part in this study. Volunteers were interviewed and 27 (12·3%) excluded for the reasons shown in Table 1. A total of 193 volunteers (177 (92%) males) were bled and immediately vaccinated intranasally with 0·5 ml. reconstituted vaccine containing $10^{7\cdot0}$ EID50 recombinant WRL 105 influenza vaccine. An 0·25 ml. volume of vaccine was administered to each nostril either as nose drops to volunteers lying down (Method A) or as a spray to volunteers who were standing or seated (Methods B, C or D) by the techniques described below:

Method A. The volunteers lay supine with their heads hyperextended.

Method B. Jencon–Repette Multi-Dose syringe with a Risdon* nasal applicator.

Method C. A Wellcome prototype disposable multi-dose spray device.

Method D. Vinelab vaccinator†.

* The Risdon Manufacturing Company, Connecticut, U.S.A.

† Vineland Laboratories Incorporated, New Jersey, U.S.A.

Table 2. *Subjects vaccinated intranasally*

	Method			
	A	B	C	D
Number	51	53	36	53
Number of males	47 (92%)	47 (89%)	34 (94%)	49 (92%)
Average age	38·9 ± 9·87	40·18 ± 10·73	39·1 ± 7·89	39·1 ± 9·73
Number providing no blood samples	2	—	—	2
Number providing no blood samples after vaccination	9 (18%)	2 (4%)	4 (11%)	2 (4%)

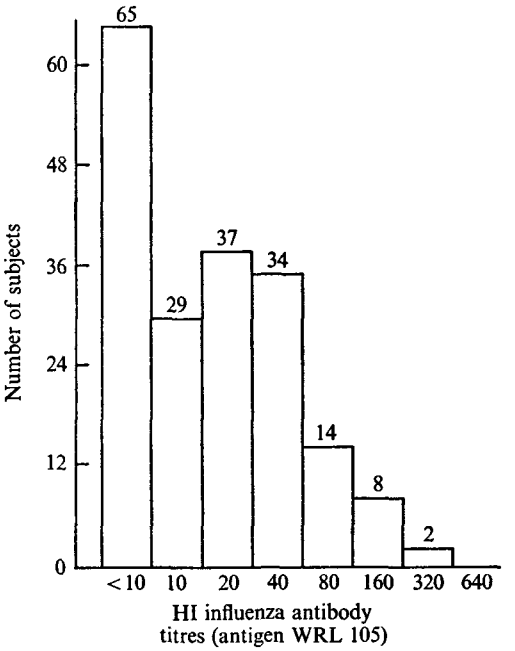

Fig. 1. Homologous HI antibody titres before vaccination.

Sprays B, C and D delivered droplets of mean diameters of 150, 300–400 and 70–75 μm. respectively.

The allocation of volunteers to the four methods of vaccination was random and the groups were equivalent in regard to age and sex (Table 2). For 1 week after vaccination volunteers completed a diary record of the occurrence of symptoms and recorded any analgesics taken for their alleviation. Three weeks after vaccination further blood samples were collected, the details of any significant reactions reviewed and volunteers completed a questionnaire on the acceptability of intranasal vaccination.

Serology

Haemagglutinating inhibiting (HI) antibody titrations were performed by the micromethod of Takatzy (1955) as modified by Sever (1962), using 0·025 ml. volumes, 8 haemagglutinating units of virus (WRL 105) and 0·6% chicken erythrocytes. Serum, virus and erythrocytes were all diluted in saline. Before testing, sera were treated with cholera filtrate (receptor destroying enzyme – RDE) to eliminate non-specific inhibitors. RDE/serum mixtures were incubated overnight at 37° C. and then heated for 30 min. at 56° C. to inactivate the RDE.

RESULTS

Serological response

Four subjects would not consent to the collection of blood samples and 17 more failed to provide samples after vaccination. The antibody titres of 189 volunteers before vaccination are shown in Fig. 1. Antibody titres of $\leqslant 20$ were found in 131 (69·3%) of volunteers. In these subjects seroconversion rates were significantly greater after the administration of vaccine by nose drops (80% – Method A) and as spray from the Jencon–Risdon device (71% – Method B) than with the other devices (Table 3). The disposable spray device (Method C) was found difficult to operate and this is the reason for the smaller number of volunteers vaccinated by this method. In general seroconversion rates fell inversely with increasing titres of antibody present before vaccination. No volunteers with initial antibody titres of $\geqslant 80$ showed a fourfold or greater antibody response.

Reactions

The vaccine was well tolerated and there were no important immediate reactions to vaccination. Reactions were compared in those who showed clear serological responses to vaccination (fourfold or greater increase in antibody titre – Group R), those who showed no serological responses to vaccination – Group N and an intermediate group who showed twofold increases in titre following vaccination – Group T – Table 4. Twofold alterations in titre are usually considered to be within the range of experimental error and of no significance. However, since there were 31 twofold increases in titre and only 6 twofold decreases in titre the reactions in those showing twofold increases in titre are shown separately. The system of recording reactions used in this study tends to over-emphasize them. However, the majority of volunteers recorded either mild, negligible or no reactions while in the remainder reactions were for the most part of no great importance. 'Severe' upper respiratory tract symptoms (nasal obstruction, nasal discharge or sore throat) and upper respiratory tract symptoms lasting 4 days or longer occurred more frequently in Group R than in Group N but this difference was not statistically significant. No differences were found for general symptoms – (headache, fever or myalgia). Nevertheless, 16 (22%) of 74 volunteers in Group R took analgesics to control symptoms in the week following vaccination in comparison with 4 (7%) of 58 volunteers in Group N, $P < 0·05$.

Table 3. *Intranasal vaccination methods*

Homologous HI antibody titre before vaccination	A (n = 40)		B (n = 51)		C (n = 32)		D (n = 49)		
	Seroconversion rate	GMT*	Seroconversion rate	GMT	Seroconversion rate	GMT	Seroconversion rate	GMT	
< 10	15/18 (83 %)	48·5	17/21 (81 %)	38·6	4/8 (50 %)	25·2	10/14 (71 %)	33·1	
⩽ 10	20/25 (80 %)	—	22/27 (81 %)	—	4/11 (36 %)	—	13/23 (57 %)	—	A, B > C $P < 0{\cdot}05$
⩽ 20	24/30 (80 %)	—	27/38 (71 %)	—	5/18 (28 %)	—	16/38 (48 %)	—	A, B > C $P < 0{\cdot}01$ A > D $P < 0{\cdot}05$
⩽ 40	27/37 (73 %)	61·6	28/47 (60 %)	48·9	6/24 (25 %)	34·2	17/40 (43 %)	36·4	A > DC $P < 0{\cdot}01$ B > C

n = number of volunteers per group. No volunteers with titres of ⩾ 80 before vaccination showed a fourfold or greater increase in titre.
* GMT, positive titres only.

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Table 4. *Comparison of reactions occurring within 7 days of intranasal vaccination with WRL 105 strain live influenza vaccine*

Antibody response to vaccination	Group R ⩾ 4-fold increase	Group N none	Group T 2-fold increase
Total/group	78	60	31
Number returning reaction forms	74 (95%)	58 (97%)	30 (97%)
Number with no reactions	29 (39%)	25 (43%)	15 (50%)
Negligible symptoms – mild 1 day's duration	8 (11%)	10 (17%)	3 (10%)
URT symptoms only Mild	12 (16%)	11 (19%)	6 (20%)
Moderate	12 (16%)	6 (10%)	2 (7%)
Severe	8 (11%)	1 (2%)	2 (7%)
All symptoms lasting ⩾ 4 days	13 (18%)	5 (9%)	4 (13%)
General symptoms Mild	13 (18%)	6 (10%)	3 (10%)
Moderate	7 (9%)	3 (5%)	5 (17%)
Severe	5 (7%)	4 (7%)	1 (3%)
All symptoms lasting ⩾4 days	4 (5%)	2 (3%)	2 (7%)
Analgesics	16 (22%)	4 (7%)	2 (7%)

In 9 subjects definite influenza-like symptoms occurred, sufficiently severe in five to result in time being lost from work. These reactions are shown in Table 5. The incidence of these reactions does not differ in Groups R, N and T.

Acceptability

All except one of 171 volunteers who completed a questionnaire found intranasal vaccination acceptable. This volunteer (Case No. 6 in Table 5) suffered influenzal symptoms which started on the 8th day after vaccination. Type A influenza virus was recovered from a throat swab collected from him at that time but its laboratory characteristics more closely resembled those of A/Port Chalmers/1/73 than the vaccine strain. Of the remaining 170, 9 (5·3%) preferred injection, 76 (44·7%) preferred nasal vaccination and 85 (50%) had no preference.

DISCUSSION

In volunteers with homologous HI antibody titres of ⩽ 20 a single intranasal dose of $10^{7\cdot0}$ EID50 recombinant WRL 105 strain live influenza vaccine administered as drops or as a spray from a Jencon syringe and Risdon applicator gave 71–80% seroconversion rates. These results are encouraging and appear to be similar to the seroconversion rates reported following the intranasal administration of other strains of live influenza vaccine (Beare *et al.* 1971). In this study the different seroconversion rates achieved by different intranasal vaccination methods indicate that the techniques and devices used are of critical importance. The administration of vaccine as nose drops was found to be a cumbersome and time consuming method ill-suited to mass administration of vaccines and the Jencon–Risdon device which gave similar seroconversion rates

Table 5. *Significant reactions*

Group R, 78 volunteers			Group T, 31 volunteers			Group N, 60 volunteers		
Symptoms	Days*	Off work	Symptoms	Days*	Off work	Symptoms	Days*	Off work
1. Influenzal symptoms	3–13	+	7. Severe nasal discharge and watery eyes	1–7	–	8. Influenzal symptoms	5–14	+
2. Influenzal symptoms	3–15	–				9. Influenzal symptoms	1–7	
3. Influenzal symptoms Secondary sinusitis treated with oxytetracycline	2–13	+						
4. Influenzal symptoms	3–5	+						
5. Influenzal symptoms	2–4	+						
6. Influenzal symptoms A strain influenza virus recovered from throat swabs collected on 8th day after vaccination. Characteristics not those of WRL 105	8–13	–						

* Days after vaccination when symptoms were recorded.

was more practical. However, it is possible that the highest seroconversion rates attainable from spray application of live influenza vaccine has not been reached in this study. Although the droplet sizes of the sprays do not appear to correlate with seroconversion rates it is important in future studies to measure the physical characteristics of sprays produced by such devices so that specifications for sprays which give the highest seroconversion rates can be established. In this study difficulties were experienced with the disposable spray and clearly devices for intranasal vaccination must be easy to use and give reproducible results in different users' hands.

Relatively few reactions were encountered after vaccination and their occurrence did not differ significantly according to serological response showing that the degree of attenuation of the strain was satisfactory. However, a few reactions of sufficient severity for volunteers to lose time from work were recorded. The study was carried out in January in a factory community in which some natural influenza was already occurring. Against this background the assessment of vaccine reaction is blurred. Further clinical trials comparing the vaccine and placebo are required to define its reactivity more closely. Ideally these trials should be carried out at a time when natural influenza is not prevalent.

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