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WRL 105 strain live attenuated influenza vaccine; comparison of one and two dose schedules

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SUMMARY

Haemagglutinating inhibiting antibody (HAI) responses were determined and clinical reactions recorded in 162 adult volunteers who received either 1 or 2 intranasal doses of $10^{7\cdot0}$ EID 50 WRL 105 strain live influenza vaccine or placebo. After administration of a single dose of vaccine significant antibody responses were obtained in 69 (70 %) of 98 volunteers with initial antibody titres of $\leqslant$ 1/20. Of the 70 volunteers who received a second dose of vaccine, 62 provided a further post-vaccination sample of serum, and only 3 (4·8 %), who had not responded to the first dose of vaccine, produced a significant antibody response.

Local, upper respiratory and constitutional symptoms were recorded more frequently after the administration of a first dose of vaccine than after placebo or a second dose of vaccine. The symptoms were of a minor nature except in one volunteer who, after the first dose of vaccine, developed influenzal symptoms followed by bronchitis.

INTRODUCTION

Parenteral administration of monovalent living attenuated viral vaccines usually elicits the development of specific circulating antibody responses in almost all susceptible individuals but a high response rate may be obtained only with difficulty when live vaccines are given intranasally. For instance, higher titres of virus were needed for immunization when rubella vaccine was administered by the intranasal than the subcutaneous route, presumably to allow for loss of vaccine into the stomach, breaching of mucosal barriers and interference by other subclinical nasal infections (Zealley, Morrison & Freestone, 1974; Hillary, 1971). The technique used to administer live vaccines intranasally has been found to bear an important direct relationship to the seroconversion rates obtained (Freestone *et al.* 1976). Nevertheless, a single intranasal dose of live influenza vaccine does not usually achieve a seroconversion rate greater than 85 % in subjects with low antibody titre even when the best techniques are employed. This study investigated whether the seroconversion rate could be significantly improved by the

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Table 1. *Volunteers in groups A and B*

	Group A	Group B
Number of volunteers included and receiving first doses of vaccine/placebo	81	81
Age range – average	18–60 years (36·65)	18–61 years (37·67)
Number of females	9 (11·1 %)	14 (17·3 %)
History during or since 1972 of		
(1) Natural influenza	30 (37·0 %)	34 (42·0 %)
(2) Vaccination against influenza	4 (4·9 %)	2 (2·5 %)

Table 2. *Design of study and number of volunteers completing each section of the trial*

		Numbers completing: Group A	Group B
Day 0	Both groups bled for titration of HI antibodies and Group A vaccinated intranasally with $10^{7\cdot0}$EID 50 WRL 105 strain influenza vaccine. Group B vaccinated intranasally with placebo	81	81
Days 1–7	Both groups completed calendar record of reactions	79	81
Day 26	Both groups bled for titration of HI antibodies	71	68
	Both groups vaccinated intransally with $10^{7\cdot0}$ EID 50 WRL 105 strain influenza vaccine	70	66
Days 27–34	Both groups completed calendar record of reactions	69	64
Day 55 (*c*)	Both groups bled for titration of HI antibodies	65*	69†

* Includes 2 volunteers who were neither bled nor vaccinated and one volunteer who was bled but not vaccinated on day 26.

† Includes 8 volunteers who were neither bled nor vaccinated and 2 volunteers who were bled but not vaccinated on day 26.

administration of a second dose of vaccine, and compared the occurrence of reactions following first and second doses of vaccine and placebo.

METHOD

Ten (5·8 %) of 172 adults who volunteered to take part in the study were not included since it was thought undesirable to vaccinate subjects with past histories of bronchitis (4), subacute bacterial endocarditis (1) or recent influenza (1). Vaccine was also not administered to 2 other volunteers who had common colds, 1 who was pregnant and one who fainted after venepuncture. The remaining 162 volunteers were randomly divided into two groups, A and B, which were subsequently found to be similar in relation to age, sex, recent past history of influenza and vaccine (Table 1). Blood samples were collected and volunteers vaccinated intranasally with WRL 105 strain live attenuated influenza vaccine (group A) or treated with placebo (group B). Second blood samples were collected 3–4 weeks

later and all volunteers were vaccinated with WRL 105 strain live attenuated influenza vaccine. Third blood samples were again collected between 3 and 4 weeks later. Reactions were recorded for 7 days after administration of each dose of vaccine or placebo on calendar record cards. The design of the trial and the number of volunteers completing each section of the trial are summarized in Table 2.

Vaccine and method of administration

WRL 105 strain live attenuated influenza vaccine is a recombinant of attenuated 280th egg passage A/Okuda/57 (H2N2) and unattenuated A/Finland/4/74 (H3N2) strains. The preparation of this recombinant and the clinical assessment of its immunogenicity, transmissibility and reactivity have been described previously (Stealey, McCahon & Freestone, 1975; Morris, Freestone, Stealey & Oliver, 1975; Moffat, Stealey, Freestone & MacDonald, 1976). The vaccine was administered intranasally as drops to give $10^{7 \cdot 0}$ EID 50 virus in an 0·5 ml. volume (0·25 ml. vaccine being administered to each nostril). Virus-free freeze-dried excipients as used in the vaccine were employed as placebo and were identical in appearance and reconstitution characteristics with vaccine.

Serological testing

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 receptor-destroying enzyme (RDE; cholera filtrate) to eliminate non-specific inhibitors. After incubation overnight at 37° C. RDE-serum mixtures were heated for 30 min. at 56° C. to inactivate the RDE.

RESULTS

Serological responses

The antibody responses and seroconversion rates obtained by the administration of a single dose of vaccine were, as expected, similar in groups A and B and are shown aggregated in Table 3. Seroconversion rates declined inversely with the titre of antibody present before vaccination. In volunteers with initial antibody titres of $\leqslant$ 1/20 a seroconversion rate of 70 % was obtained. Treatment with placebo 1 month previously had, as expected, no effect on the seroconversion rate in the volunteers in group B. However, 4 (5·3 %) of 76 (3 of 68 volunteers bled 3 weeks, and 1 of 8 volunteers bled 6 weeks) were found to have a fourfold or greater increase in antibody after administration of placebo.

A second dose of vaccine was administered to volunteers in group A and produced a fourfold or greater antibody response in only 3 (4·8 %) of 62. However, in group A, 19 volunteers showed twofold increases and 11 volunteers two-fold decreases in antibody titre resulting in a modest increase in geometric mean titre.

Table 3. *Serological responses to intranasal administration of first dose of* $10^{7\cdot0}$ *EID*50 *WRL* 105 *strain live influenza vaccine groups A and B*

HI titre before vaccination	Seroconversion rate	GMT
<10	39/47 (83·0%)	34·9†
10	15/23 (65·2%)	46·5
20	15/28 (53·6%)	64·0
40	5/20 (25·0%)	74·6
80	1/5 (20·0%)	91·9
$\geqslant 160$	0/9 (0·0%)	—‡

Conversion rate in volunteers with titres before vaccination of $\leqslant 20$ = 69/98 (70·4%)
† Positive titres only
‡ Not calculated.

Reactions

Over 95% of volunteers recorded details of reactions after both doses of vaccine or placebo. In analysing these results, reactions at their most severe were used in the evaluation of the data. Table 4 shows the frequency of local symptoms referable to the upper respiratory tract (nasal obstruction, nasal discharge or sore throat), general symptoms (headache, fever or myalgia) and the use of analgesics in the week after treatment with vaccine or placebo. A first dose of vaccine was associated with a somewhat greater consumption of analgesics and a greater frequency of local and general symptoms which were of longer duration than occurred following treatment with placebo or a second dose of vaccine. Statistically significant differences ($P < 0{\cdot}05$) were obtained between a first dose of vaccine in group A and placebo (group B) for: 'all reactions' and for the occurrence of, and the duration of local reactions. Other differences after first and second doses of vaccine were not statistically significant.

One volunteer in group A developed influenza 36 hr. after administration of the first dose of vaccine. This was followed by secondary bronchitis which was treated with antibiotics. He was in bed for 14 days.

DISCUSSION

In volunteers with antibody titres before vaccination of $\leqslant 1/20$ a single dose of WRL 105 strain live attenuated influenza vaccine administered as drops elicited fourfold or greater antibody responses in 70%. Little increase in seroconversion rate was obtained after the administration of a second dose of vaccine indicating that these volunteers were protected against infection with homologous strains of influenza either by the administration of the first dose of vaccine or by pre-existing antibody induced by natural infection. These serological results correlate with the lower incidence of reactions in volunteers in group A after the administration of the second dose of vaccine.

It is not possible to determine whether the increase in antibody in four

Table 4. *Reactions recorded following administration of first and second doses of vaccine and placebo*

	Group B Placebo control	Group A First dose of vaccine	Group B First dose of vaccine	Group A Second dose of vaccine
Total number per group	81	81	66	70
Number returning reaction forms	81 (100 %)	79 (97·5 %)	64 (97·0 %)	69 (98·6 %)
Number recording symptoms	32 (39·5 %)*	44 (56·7 %)*	24 (37·5 %)	18 (26·1 %)
Analgesics taken	12 (14·8 %)	13 (16·6 %)	11 (17·2 %)	4 (5·8 %)
Local symptoms:				
Mild	13 (16·0 %)	22 (27·8 %)	8 (12·5 %)	13 (18·8 %)
Moderate	9 (11·1 %) ††	13 (16·5 %) ††	4 (6·3 %)	4 (5·8 %)
Severe	3 (3·7 %)	6 (7·6 %)	6 (9·4 %)	—
Lasting ⩾ 4 days	6 (7·4 %)*	15 (19·0 %)*	7 (10·9 %)	5 (7·2 %)
General symptoms:				
Mild	13 (16·0 %)	16 (20·3 %)	6 (9·4 %)	3 (4·3 %)
Moderate	5 (6·2 %)	9 (11·4 %)	7 (10·9 %)	4 (5·8 %)
Severe	3 (3·7 %)	6 (7·6 %)	5 (7·8 %)	2 (2·9 %)
Lasting ⩾ 4 days	2 (2·5 %)	5 (6·3 %)	6 (9·4 %)	1 (1·4 %)
Negligible symptoms	4 (4·9 %)	6 (7·6 %)	7 (10·9 %)	6 (8·7 %)

* $P < 0{\cdot}05$. † $P < 0{\cdot}01$.

volunteers in group B after the administration of placebo is the result of natural infection, present in some employees at the time that the trial was carried out, or of transmitted infection with vaccine virus. However, no evidence of spread has been found in three other studies specifically designed to assess transmissibility (Morris *et al.* 1975; Moffat *et al.* 1976; D. S. Freestone, personal communication). It is of interest that fewer reactions followed the administration of the second dose of vaccine than followed treatment with placebo. There is no certain explanation for this unexpected finding. However, it seems possible that volunteers might have been less concerned or less vigilant about reactions after the second dose of an experimental vaccine than after the first. There is no evidence that volunteers reacting to a first dose of vaccine failed to come forward for a second dose or that the same volunteers reacted after both doses.

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