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The efficacy of live and inactivated vaccines of Hong Kong influenza virus in an industrial community

A report to the Medical Research Council Committee on Influenza and other respiratory virus vaccines.*

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SUMMARY

Intranasal vaccines of inactivated or living attentuated A2/Hong Kong influenza viruses were compared for clinical acceptability, serological effects and protective efficiency against natural epidemic influenza in a large industrial and clerical population.

Neither vaccine resulted in any significant untoward side-effects. The serum haemagglutination-inhibiting (HI) antibody response within 1 month of vaccination was similar with both vaccines; approximately 50% of those with little or no pre-vaccination antibody developed 4-fold or greater rises in titre. The effect of the antigenic potency of the vaccines and the prior immunological experience of the population is discussed. Volunteers given live vaccine showed a 2·2-fold lower incidence of clinical influenza than those given killed vaccine in a natural epidemic 16 months after vaccination.

INTRODUCTION

In previous small-scale clinical trials (e.g. Beare, Hobson, Reed & Tyrrell, 1968) living influenza virus vaccines given intranasally to adult volunteers induced an immune response, especially in terms of circulating antibody against the viral haemagglutinin (HA) antigen, which conferred protection against intranasal challenge infection with a homologous virus strain 2–4 weeks after vaccination. The live vaccines were easily administered by nasal drops or spray, appeared to evoke few or no clinical signs or symptoms of infection, and the amount and duration of virus excretion was low.

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The purpose of the present trial was to determine (*a*) whether live intranasal vaccines are equally acceptable under field conditions in large industrial and clerical populations, (*b*) whether killed intranasal vaccine would be equally immunogenic but less reactogenic, as suggested by the studies of Waldman, Small & Rowe (1970) and (*c*) whether significant protection could be demonstrated against any natural influenza epidemic occurring in the community in the subsequent two years.

MATERIALS AND METHODS

In November 1970, 3 schedules of vaccination were compared in randomly chosen groups of volunteers in the headquarters staff of the Midland Region of the Central Electricity Generating Board in Solihull, Warwickshire. Group 1 received two intranasal doses, at fortnightly intervals, of a live attentuated strain of A2/Hong Kong virus. Group 2 were given placebo material in the first session, to facilitate the investigation of clinical responses to the live vaccine, and a single dose of live vaccine two weeks later, at the time Group 1 were receiving their second dose. Group 3 were given two intranasal doses at fortnightly intervals of a killed vaccine containing the recombinant virus X31 which is antigenically identical with A2/Hong Kong (Kilbourne *et al.* 1971). Details of these vaccines are given below. A total of 450 H.Q. staff were vaccinated; a detailed questionnaire on clinical reactions to the vaccine was given to each volunteer, and blood samples were obtained before and after vaccination from 147 of them.

An extended but somewhat simplified trial of the same vaccines was also conducted in November 1970 in the generating stations of C.E.G.B. Midland region. The main purpose was to allow larger-scale investigations of the protective effect of the vaccines in the event of an influenza epidemic, and no studies were made of the serological effect of the vaccines or of clinical side-effects to them; 1836 volunteers from a total working population of 15,592 were randomly allocated to receive either a single dose of live Hong Kong vaccine or a single dose of killed X31 vaccine. All sickness absence due to respiratory infection in each vaccinated group, and in the total H.Q. and station population, was evaluated over the periods November to March (pay weeks 35–52) of 1970–1 and 1971–2.

All the vaccines in the present trial were made and generously given by Evans Medical Limited, Speke, Liverpool. The live vaccine was prepared from a seed pool of an inhibitor-resistant strain A2/HK/1/68/EG.972 which had previously undergone serial allantoic passages in chick embryos in the presence of horse serum prior to terminal dilution purification in RIF-free eggs. A single allantoic fluid pool was made in RIF-free eggs, purified by differential centrifugation and freeze-dried in 1 ml. amounts (ten doses) in ampoules in a freeze-drying medium routinely used in commercial measles vaccine manufacture. The vaccine was freshly reconstituted in distilled water just before use; the virus titre of each ampoule was $10^{7\cdot2}$ EID_{50} (egg infective doses) per ml. The placebo vaccine consisted of freeze-drying medium only, dispensed in ten-dose ampoules, and was identical with the live vaccine except for a 'blind' letter-coding. The killed vaccine was an aqueous suspension of X31 virus, inactivated with 1/1000 β-propiolactone; each 0·1 ml.

Table 1. *The effect of intranasal Hong Kong influenza virus vaccines on homologous HI antibody titres in serum*

		Vaccine group 1	2	3
Total of volunteers tested		53	43	51
No. without pre-vaccine antibody (< 6)		18 (34%)	15 (35%)	16 (31%)
No. with ⩾ fourfold rise in titre		12 (23%)	1 (2·3%)	12 (23·5%)
No. with twofold rise in titre		7 (13%)	2 (4·6%)	14 (27%)
No. with ⩾ twofold *fall* in titre (estimate of technical error in above)		0	2	0
No. of ⩾ fourfold rises in persons of different pre-vaccine titres (% of total at each initial level)	< 6	6 (34%)	1 (8%)	6 (36%)
	6	5 (80%)	0	2 (66%)
	12	1 (12%)	0	3 (34%)
	24	0	0	1 (16%)
	48	0	0	0
G.M.T. of whole group	Pre-vaccine	9·3	9·1	11·5
	Post-vaccine	17·8	9·8	25·1

Vaccination schedules	1st dose	2nd dose
Group 1	Live HK	Live HK
Group 2	Saline	Live HK
Group 3	Inactivated HK	Inactivated HK

Interval between doses = 2 weeks.
2nd serum 2 weeks after 2nd dose.

dose contained 1500 HAU (approx. ten times more HA units than the dose of live vaccine). Each type of vaccine was given in a 0·1 ml. dose, placed dropwise high into the volunteer's nostrils from disposable plastic syringes fitted with a 3-in. 18-gauge metal cannula. The patient was instructed to keep his head tilted back, and sniff up the inoculum for one minute after instillation, and was told not to blow his nose for one hour. Separate operators and equipment in well-segregated areas of the clinic were used for each vaccine.

Serum samples were stored at $-20°$ C., and titrated in pairs for HI antibody, after treatment with cholera filtrate to destroy non-specific virus inhibitors. The homologous live vaccine strain of HK virus was used throughout; 4 HA units of virus were incubated with serum dilutions for 1 hr. at room temperature before adding a 1% suspension of fowl red cells. The general performance of the test was as for the W.H.O. plastic plate method (W.H.O. Report, 1953).

RESULTS

Serological responses to vaccination

Details of the effect of each vaccine on serum HI antibody titres are shown in Table 1, from which it is apparent that almost all increases in titre were in those initially without detectable antibody or with pre-vaccine titres of 24 or less. Two doses of live or killed vaccine were equally effective, inducing fourfold or greater

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Table 2. *The clinical effects of intranasal vaccines of Hong Kong influenza virus*

Clinical symptoms notified	No. of volunteers with symptoms within 7 days of first dose		
	Group 1, live vaccine	Group 2, placebo	Group 3, inactivated vaccine
Sneezing	28	21	21
Stuffy or running nose	31	39	38
Sore throat or cough	30	19	21
Headache	23	24	22
General malaise, or aching limbs	19	25	21
Off work, with any of above symptoms	3	3	4
Total no. of volunteers with one or more symptoms	54	58	57
Total no. in each group	107	107	124

Completed questionnaires were returned by 75% of those vaccinated.

rises in titre in 23% and 23·5% of volunteers respectively. A single dose of live vaccine appeared to have no significant effect on HI titres, but the post-vaccination HI test in this group was only two weeks after their single dose of vaccine whereas, in the other two groups, the interval between the first dose of vaccine and the post-vaccination sample was four weeks. Hence, if the immune response to vaccine is mainly attributable to the first dose, as in our previous trials (Beare *et al.* 1969), rises in titre would be more likely to have occurred in Groups 1 and 3 than in Group 2, at the time of sampling.

Clinical effects of vaccination

Questionnaires were returned by 338 (75%) of H.Q. staff after their first dose of vaccine. The analysis of responses (Table 2) shows that half the volunteers reported one or more symptoms, but that neither of the vaccines gave an enhanced or different pattern of reactions compared with the placebo material. Thus the physical or subjective effects of intranasal inoculation seem of greater importance than the content of the inocula. Similarly, after the second dose of vaccine there was no significant difference in the frequency of symptoms in those in Group 1 who had already had a previous dose of live vaccine and those in Group 2 receiving vaccine for the first time.

The protective effects of vaccination

The sickness absence of all volunteers and non-vaccinated personnel on all the stations in the trial was recorded by computer from the diagnosis given in the medical certificate from the patient's family doctor.

The number of illnesses attributed to influenza, coryza and bronchitis was calculated separately. Since many respiratory illnesses, even mild attacks of influenza, might cause a loss of less than 3 working days, computer data on short 'uncertified illness' were also examined. Data for the whole study population, including H.Q.

Table 3. *Differences in the incidence of respiratory illness in vaccinated and unvaccinated personnel*

(Data for categories in which there were no significant differences at any time in either winter between any of the groups have been omitted. Group L, live HK vaccine, 910 persons. Group K, killed HK vaccine, 926 persons. Group U, no vaccine, 13,756 persons.)

Data on time off work in each pay-week period	Sole category of difference in year of observation over period 1 November–1 April					
	1970–1, Bronchitis			1971–2, Influenza		
	L	K	U	L	K	U
Weeks 35–9						
No. ill	8	6	134	7	7	68
% ill	0·8	0·6	1·1	0·8	0·8	0·5
Days absent/person	7·5	6·5	12·3	5·1	8·0	6·4
Weeks 40–3						
No. ill	14	12	243	8	10	123
% ill	1·5	1·3	1·9	0·9	1·1	0·9
Days absent/person	7·0	11·1	11·0	6·9	6·1	6·7
Weeks 44–7						
No. ill	8	6	183	8	21	268
% ill	0·8	0·6	1·4	0·9	2·3	1·9
Days absent/person	6·7	9·5	10·8	7·7	6·2	7·0
Weeks 48–52						
No. ill	3	7	149	9	15	195
% ill	0·3	0·7	1·2	1·0	1·6	1·4
Days absent/person	5·7	6·4	9·7	10·3	5·9	6·5

staff, were reviewed collectively simply as live vaccine versus killed vaccine versus unvaccinated personnel. The errors inherent in comparing those who volunteer for vaccination with those who do not are well recognized; however, since the nature of the trial precluded a group of volunteers given only placebo, it was felt worthwhile to include this extra statistic.

Data for 1970–1

In the first winter after vaccination, Hong Kong virus isolations were reported only very sporadically by the Public Health Laboratory Service in various parts of the country, and there were no frank epidemics. The computer data showed a constant low incidence of 'influenza', coryza and uncertified illness in all groups with no significant differences between them. On the other hand, illnesses certified as 'bronchitis' (see Table 3) were significantly less in each of the two vaccinated groups than in unvaccinated persons in pay weeks 44–7 ($\chi^2 = 5{\cdot}5$, $P < 0{\cdot}02$) and weeks 48–52 ($\chi^2 = 6{\cdot}8$, $P < 0{\cdot}02$). The differences between those given live and those given killed vaccine were not significant.

Data for 1971–2

There was a sharp increase in HK virus influenza throughout England early in December 1971, which reached a peak late in February 1972, and did not disappear

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until mid-April 1972. Although no virological confirmatory tests were made in the C.E.G.B. population, it is reasonable to assume that the increase in illnesses certified as influenza in weeks 44–7 (see Table 3) were part of the HK epidemic in the general local population. In this period 268 (1·9 %) of the unvaccinated and 21 (2·3 %) of the group given killed vaccine had an influenzal illness as compared with only 8 (0·9 %) of those given live vaccines; these differences are statistically significant overall ($\chi^2 = 5{\cdot}9$, $P < 0{\cdot}05$). The sickness rate was significantly less after live vaccine than either killed vaccine ($\chi^2 = 5{\cdot}7$, $P < 0{\cdot}02$) or no vaccine ($\chi^2 = 5{\cdot}4$, $P < 0{\cdot}02$) whereas there was no significant difference between killed vaccine and no vaccine. In this second winter, unlike 1970–1, the incidence of bronchitis was not apparently influenced by prior vaccination.

DISCUSSION

Before vaccination, 67 % of volunteers had already acquired serum HI antibody against HK virus. The effect of live vaccine, as in previous trials (Hobson, Curry, Beare & Ward-Gardner, 1972) was almost entirely to immunize the previously non-immune rather than to boost the titre of those who already had prior experience. The serological response was as good with the inactivated as with the live intranasal vaccine, as indicated above. It may be important that on this occasion the antigenic mass of the original inoculum was ten times greater in the inactivated than in the live vaccine. It is also possible that the apparent improvement over results in our previous trials is due to the prior experience of HK antigen of most of the present population, perhaps even in some to those with no detectable serum antibody just before inoculation. When similar inactivated intranasal HK vaccine was given in 1968, before there had been extensive natural spread of the virus, the serological response was much less than after parenteral HK vaccine (Hobson *et al.* 1970), and there was no protection against the first HK epidemic, which occurred 2–4 months after vaccination (Tyrrell, Buckland, Rubenstein & Sharpe, 1970). Similarly, the serological and protective effects of HK-killed intranasal vaccines recently reported by Waldman & Coggins (1972) were considerably better than in their earlier trials (Waldman *et al.* 1970) when HK virus was new and the population less primed.

The protective effect against influenza of the live vaccine was greater than that of killed intranasal vaccine, even though they had produced comparable serological responses shortly after administration. It is thus probable that live vaccine induced a higher and more prolonged degree of local immunity in the respiratory tract.

It is more difficult to explain the apparent effect of both vaccines in reducing the incidence of bronchitis in 1970–1. It is probable that, in periods when influenza viruses are only sporadically circulating, many short individual illnesses caused by these agents are designated as colds or bronchitis, chills etc. Influenza vaccines may thus appear to protect against apparently unrelated syndromes (e.g. Reports 1959; 1964). Conversely, in periods when influenza is known to be epidemic, there is a tendency to certify bronchitis and febrile colds as influenza. In small controlled trials it is obviously possible to minimize these errors by

obtaining virological proof of all infections, or by the substitution of a deliberate challenge infection for a natural epidemic. In field trials of the present nature more precise data than those described here are only likely to be obtained by greatly increasing the scale of the trial or by waiting for the emergence of a new virus serotype to which the majority of unvaccinated people would be highly susceptible.

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