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A controlled trial of inactivated monovalent influenza A vaccines in general practice

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SUMMARY

A trial of influenza A vaccines in general practice is described. Five hundred and seven subjects were vaccinated with either inactivated monovalent A/Hong Kong vaccine, A/England vaccine or influenza B vaccine as control. Local reactions were noted in 24% and general reactions in 12% of patients. Antibody titres in serum were measured by haemagglutination inhibition (HI) and complement fixation (CF) tests in 465 subjects. The influenza vaccines produced substantial increases in both homologous and heterologous antibodies as measured by the HI test and a comparatively poor response as measured by the CF test. Although clinical influenza was confirmed in only a few cases, there was serological evidence of significant subclinical infection in the control group.

INTRODUCTION

Despite widespread use there is still disagreement about the place of influenza vaccine in the control of influenza. The majority of vaccine trials have involved defined populations such as Service personnel, industrial workers, university students and pupils in boarding schools. Few studies have been reported from family practice. This paper presents such a study. With the collaboration of members of the Royal College of General Practitioners a trial was arranged to assess the efficacy of inactivated monovalent influenza A vaccines against expected influenza in the winter of 1972/73. The investigation included an adequate control group inoculated with influenza B vaccine, and laboratory facilities for the virological investigation of suspected cases and the measurement of antibody response to the different vaccines. In the event there was little influenza in Leicester during the winter and it was not possible to assess the protective efficacy of the vaccines. However, the vaccination reactions, antibody response and virus isolations observed in the trial are described below.

MATERIAL AND METHODS

The study was carried out in patients from 25 general practices in Leicestershire who had agreed to participate and to give the necessary blood samples. A first sample was withdrawn towards the end of November 1972 when patients were given one of three monovalent vaccines by random allocation. A second sample was withdrawn three weeks later and a final sample at the end of the following March. Participants were asked to report any symptoms of influenza and those who did so had a throat swab taken for virological examination. Acute and convalescent blood samples were also requested.

Vaccines

The three inactivated monovalent vaccines which were prepared by Evans Medical Limited and supplied by the Epidemiological Research Laboratory, Colindale, were as follows:

1. Vaccine A/HK contained A/Hong Kong/1/68(H3N2) (recombinant X31).
2. Vaccine A/Eng contained A/England/42/72(H3N2) (recombinant XPR8).
3. Vaccine B contained B/Victoria/98926/70.

Administration

The identity of each vaccine was concealed by a code number known only to the laboratory. Each vaccine was given intramuscularly, the A vaccines in a dose of 600 i.u. and the B vaccine in a dose of 400 i.u. The vaccines were allocated according to day of birth; those born on the 1st–10th day of any month received A/HK, on the 11th–20th B, and on the 21st–31st A/Eng vaccine.

Reactions to vaccination

On returning about three weeks later for the post-vaccination sample the patient was asked about local and general reactions. These were recorded as nil, moderate or severe. Locally a moderate reaction was characterized by slight swelling and aching at the site of injection. A reaction was recorded as severe when the arm became painful, red and swollen. Constitutionally a moderate response was characterized by slight pyrexia and mild aching of the back and limbs and a severe reaction by influenza-like symptoms. In the event of illness the date and details of symptoms were recorded by the general practitioner.

Serological methods

HI tests were carried out in microplates using 0·025 ml volumes of serum and equal volumes of virus containing eight haemagglutinating units. Each serum was tested for HI antibodies against A/Hong Kong/1/68(H3N2), A/England/42/72(H3N2) and B/England/21/68 viruses.

CF tests were carried out according to the method described by Bradstreet & Taylor (1962) modified by the use of a microtitre technique.

Table 1. *Age and sex distribution of vaccinated patients*

Sex	Age						Total
	< 25 years	25–34 years	35–44 years	45–54 years	55–64 years	⩾ 65 years	
Male	15	35	50	43	27	16	186 (40 %)
Female	23	63	72	58	35	28	279 (60 %)
Total	38	98	122	101	62	44	465

Virus isolation

Throat swabs in transport medium were inoculated within 24 hr of collection in rhesus monkey kidney tissue culture cells supplied by the Biological Standards Division of the National Institute of Medical Research. The cells were maintained in mixture '199' without serum. The monkey kidney cells were tested for haemadsorption after 2, 4, 7 and 14 days, and examined for the presence of cytopathic effect. Each specimen was also inoculated in the Bristol line of HeLa cells and human embryo lung cells for the isolation of other respiratory viruses.

RESULTS

A total of 507 patients from the 25 practices entered the trial. Any patient who failed to supply at least two blood samples was excluded and consequently 465 patients qualified for inclusion in the analysis.

All but six of the patients were adults. The age and sex distribution is shown in Table 1. There were more females than males, but the age distribution was similar for both.

One hundred and forty one (30 %) patients were given A/HK vaccine, 155 (33 %) B vaccine and 169 (36 %) A/Eng vaccine. The slight excess in the A/Eng group was expected because of the extra days of birth allocated to this group. The method of allocation appears to have been successful since the three groups were of comparable size with similar age and sex composition and also similar pre-vaccination antibody titres.

Reactions to vaccination

Information on local reactions was recorded for all but 20 of the 465 patients. Ninety-seven (22 %) reported mild, and 12 (3 %) severe local reactions, which were slightly more frequent in the younger age groups. Information on general reactions was recorded for all but 15 of the patients. Forty-two (9 %) reported mild and 11 (2 %) severe general reactions. The rates were similar in all age groups. Each of the three vaccines gave similar reaction rates.

HI antibody results

Pre-vaccination results

Many patients had HI antibody to A/HK at the time of vaccination (Table 2). Of 463 patients 306 (66 %) had antibody titres of 20 or more. In contrast only 137 (30 %) had antibody to A/Eng. In 264 patients the A/Eng antibody titre was

Table 2. *Pre-vaccination HI antibody titres to A/HK and A/Eng*

			A/HK antibody Number of patients with titre					
			⩽ 10	20	40	80	⩾ 160	Total
A/Eng antibody	Number of patients with titre	⩽ 10	153	77	54	28	14	326
		20	3	6	27	18	10	64
		40	1	2	8	13	8	32
		80	—	—	1	3	15	19
		⩾ 160	—	1	1	1	19	22
		Total	157	86	91	63	66	463

Table 3. *Changes in HI antibody titre to A/HK and A/Eng in* 137 *patients after inoculation with A/HK vaccine*

	HI antibody to A/HK		HI antibody to A/Eng	
Antibody titre	Before vaccination	After vaccination	Before vaccination	After vaccination
⩽ 10	52	5	101	20
20	25	3	13	13
40	21	7	10	22
80	21	29	6	25
160	8	21	2	28
320	3	29	3	10
640	4	21	1	14
⩾ 1280	3	22	1	5
Total patients	137	137	137	137
Geometric mean titre	38	310	15	110

lower than their A/HK titre, in 189 patients it was the same and in only 10 was it higher. Despite this tendency for antibody titres to A/Eng to be lower there was a correlation with the A/HK antibody since patients with high A/HK titres were more likely to have high A/Eng titres than those with low A/HK antibody.

The number of patients with HI antibody to influenza B was smaller, only 23 % having titres of 20 or more.

An analysis not presented in the table showed that the proportion of patients with A/HK antibody was similar in all age groups, whereas the proportion of patients with antibody to A/Eng increased slightly with age – in the ⩾ 65 age group 34 (45 %) had antibody to A/Eng, a proportion which was significantly greater than the 30 % at all ages ($P < 0{\cdot}02$). Antibody to influenza B was proportionally greater (41 %) in the group under 25 years old ($P < 0{\cdot}001$).

Post-vaccination results

Of the 141 patients who received A/HK vaccine, 137 had pre- and post-vaccination samples tested. All but 5 of the 52 patients without A/HK antibodies before vaccination (i.e. titres of 10 or less) developed A/HK antibody after vaccination (table 3). In most patients with pre-vaccination antibody the titre rose substantially. The average increase in titre was about 8-fold; the geometric mean titre

Table 4. *Changes in HI antibody titre to A/HK and A/Eng in 164 patients after inoculation with A/Eng vaccine*

Antibody titre	HI antibody to A/HK		HI antibody to A/Eng	
	Before vaccination	After vaccination	Before vaccination	After vaccination
⩽ 10	51	11	111	5
20	33	9	24	12
40	32	8	13	15
80	16	13	6	18
160	16	22	8	32
320	9	27	1	22
640	5	31	—	23
⩾ 1280	2	43	1	37
Total patients	164	164	164	164
Geometric mean titre	43	370	16	310

Table 5. *Increase in influenza A antibody titres after vaccination with A/HK and A/Eng vaccines in patients with and without pre-vaccination antibody*

Presence (+) or absence (−) of pre-vaccination HI antibody to (pre-vaccination GMT)		Number vaccinated	Vaccine given	Increase in titre to	
				A/HK	A/Eng
A/HK− (GMT < 10	A/Eng− GMT < 10)	51	A/HK	× 16	× 8
A/HK+ (GMT 56	A/Eng− GMT < 10)	50	A/HK	× 8	× 16
A/HK+ (GMT 172	A/Eng+ GMT 81)	35	A/HK	× 2	× 2
A/HK− (GMT < 10	A/Eng− GMT < 10)	51	A/Eng	× 12	× 16
A/HK+ (GMT 60	A/Eng− GMT < 10)	60	A/Eng	× 12	× 64
A/HK+ (GMT 145	A/Eng+ GMT 60)	53	A/Eng	× 6	× 6

(GMT) rose from 38 to 310. Vaccination with A/HK also induced the development of A/Eng antibody (Table 3). Thus among the 137 patients 101 had no pre-vaccination antibody to A/Eng and of these 81 developed antibody. Again there was an average 8-fold increase; the GMT rose from 15 to 110.

Of the 169 patients who received A/Eng vaccine 164 had pre- and post-vaccination samples tested. All but five of the 111 patients without A/Eng antibody before vaccination developed antibody after vaccination, and in most patients there was a substantial increase in titre (Table 4). The average change was nearly 20-fold; the GMT rose from 16 to 310. Vaccination with A/Eng was also associated with the development of A/HK antibody (Table 4). Thus among these 164 patients there were 51 without pre-vaccination antibody to A/HK and all but 11 of these developed antibody after vaccination. There was an average increase of just over 8-fold; the GMT rose from 43 to 370.

Table 6. *Changes in HI antibody to B in* 151 *patients after inoculation with B vaccine*

Antibody titre	HI antibody to B: Before vaccination	HI antibody to B: After vaccination
⩽ 10	114	35
20	22	22
40	9	29
80	5	25
160	1	20
320	—	9
⩾ 640	—	11
Total patients	151	151
Geometric mean titre	13	65

The antibody response to each vaccine was affected by the presence or absence of antibody to A/HK and A/Eng before vaccination. The responses are summarized in Table 5 which shows the post-vaccination changes in titre according to the status of pre-vaccination antibody. It is clear that each vaccine produced substantial increases in both homologous and heterologous antibodies. The increases were less in the groups of patients who already had antibody to both antigens and whose pre-vaccination GMT of A/HK antibody was the highest. However, the A/Eng vaccine tended to be more effective in producing antibody to the A/Eng strain.

It is of interest that the presence of antibody to A/HK in the absence of antibody to A/Eng before vaccination was associated with a significantly better response to A/Eng antibody. This feature was seen after the administration of each vaccine.

Response to the vaccine containing the B strain was less than to vaccines containing the A strains. Of the 155 patients allocated B vaccine 151 had pre- and post-vaccination samples tested (Table 6). There were 114 patients without pre-vaccination antibody and 35 still had no antibody after vaccination. The average change was just over 4-fold; the GMT rose from 13 to 65.

End-of-trial results

Antibody titres tended to fall slightly during the three months or so between the post-vaccination and final samples. Fig. 1 shows average antibody titres to A/Eng for each of the three vaccination groups at the three stages of the trial. A slight increase in average titre among patients receiving B vaccine suggests that some of these patients were infected with A/Eng virus around the time of the start of the trial; of the 155 patients in the B vaccination group 15 showed pre- to post-vaccination 4-fold rises in titre to A/Eng, and three more patients showed similar rises during the post-vaccination to end-of-trial period.

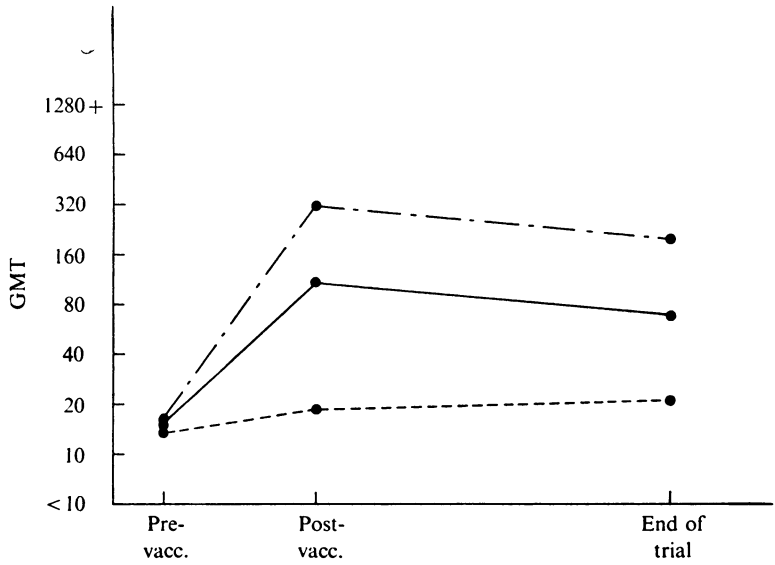

Fig. 1. Average HI antibody titres to A/England at each stage of the trial, according to vaccine given. Vaccine given: ———, A/HK; — — —, A/Eng; – – – – –, B.

CF antibody results

Many patients had CF antibody to influenza virus at the time of vaccination. Of the 463 entry specimens tested 302 (65 %) had titres of 8 or more to A antigen and 245 (53 %) to B antigen.

Of the 141 patients given A/HK vaccine 137 had pre- and post-vaccination tests for CF antibody. These included 36 with no antibody to A antigen before vaccination (i.e. titres of 4 or less) and 25 of these still had no antibody three weeks after vaccination. Similarly with the 169 patients given A/Eng vaccine 158 had both samples tested; 55 had no pre-vaccination antibody and 44 of these did not acquire antibody after vaccination. Among all patients receiving an A vaccine the average increase in titre was barely 2-fold; the GMT rose only from 21 to 34.

One hundred and fifty one patients given B vaccine had pre- and post-vaccination samples tested by CF. There were 63 patients without pre-vaccination antibody to B antigen and 25 of these still had no antibody after vaccination. The average change in antibody was about 2-fold ; the GMT rose from 11 to 20.

Only slight falls in average of CF antibody were seen over the post-vaccination to end-of-trial period. Fig. 2 shows average A antibodies at the three stages of the trial in each of the three vaccine groups. The group receiving B vaccine, which acts as a control group, showed virtually no change over the whole period.

Comparison of HI and CF antibodies

Table 7 shows responses as measured by the two methods in patients receiving either of the A vaccines. Fewer patients showed response to vaccination by the CF tests as compared with HI tests. Of 310 patients whose paired sera were tested by both methods, only 47 (15 %) showed a 4-fold or greater rise in titre by the CF test as compared with 235 (76 %) by the HI test.

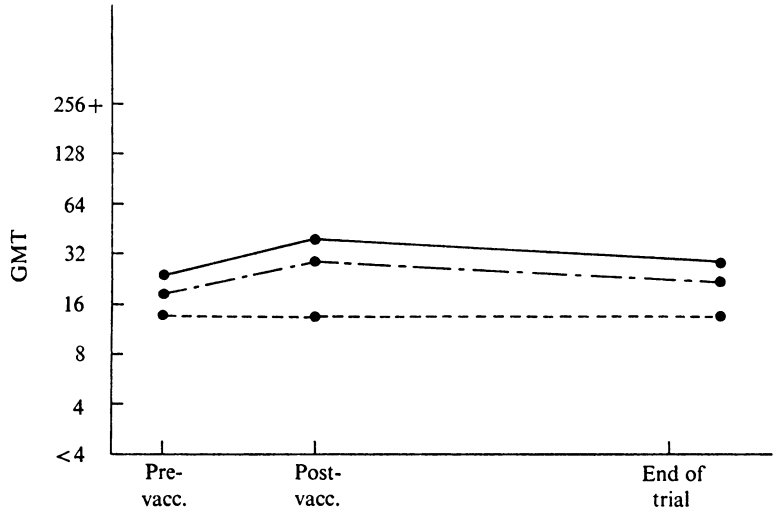

Fig. 2. Average CF antibody titres to influenza A at each stage of the trial, according to vaccine given. Vaccine given: ———, A/HK; — - —, A/Eng; - - - - - -, B.

Table 7. *Increase in antibody titre in* 301 *patients against influenza A virus as measured by CF and HI tests*

Increase in titre	CF test	HI test
No increase	174	31
× 2	80	35
× 4	27	41
× ⩾ 8	20	194

Patients reporting 'influenza-like' illnesses

Thirty-two (7 %) of the 465 patients reported an influenza-like illness during the period of the trial. Only four cases were confirmed by laboratory examination to be A/Eng infections; three gave positive isolations from throat swabs and the fourth had an 8-fold rise in CF antibody. Two of these cases had received B vaccine, one A/HK vaccine and one A/Eng vaccine. However, in this last patient symptoms had already begun when the vaccine was given. All four cases were reported in November and December.

The remaining 28 cases occurred at intervals throughout the period of the trial. Nine of these had received A/HK vaccine, 11 A/Eng vaccine and 8 B vaccine. These patients were representative of all patients in the trial in respect of age and sex distribution and pre-vaccination titres. Only four of these 28 cases yielded virus from the throat swab. The viruses were identified as rhinovirus (2), respiratory syncytial virus (1) and herpes simplex virus (1). Serological examination of acute and convalescent sera has so far been confined to influenza antigens.

Symptoms were recorded and some differences were observed between the probable influenza cases and the rest, but the numbers are very small and not statistically significant. All four influenza cases reported aching limbs, headache and cough and three reported fever and rigors.

DISCUSSION

Influenza vaccines have frequently been assessed by field trial but there is still disagreement about their place in influenza control. Usually influenza vaccine trials are made in factory populations or in other defined communities. The present study, however, was made in family practice. It incorporated the features essential for an adequate assessment of influenza vaccine – a control group chosen by an effective random method and facilities for laboratory examination of cases of influenza-like illness. Laboratory facilities were also available to examine the antibody response to the vaccines used.

In the present study there was a wide age distribution in volunteer patients; the greater proportion of females appears to be similar to that usually found in surgery consultations. Of this population a large proportion must have been infected with A/HK influenza since two-thirds had HI antibody to this strain. A considerable, though smaller proportion had also been infected with the A/Eng strain. The proportion with this antibody – 30 % – was similar to that found by Pereira *et al.* (1972) and presumably reflects the presence of the virus in England during the previous months. However, it is difficult to see how recent infection could account for the greater proportion of older people with antibody to the virus. This excess in the older patients was considerable and attained statistical significance. This finding suggests that the older group may have been infected with a strain antigenically similar to the A/Eng strain earlier in life. If this is so such strains have presumably circulated in England in the past, before identification of influenza strains became possible.

In contrast to the age distribution of A/Eng antibody, HI antibody to the 1968 influenza B strain was more common in persons under 25. Influenza B tends to be seen most commonly in the young, and so the older group in this trial had presumably escaped infection with this strain.

Like most inactivated influenza vaccines, the two monovalent A vaccines produced a good HI antibody response to the homologous virus strain. Each vaccine, however, also produced a good response to the heterologous strain. Both virus strains clearly include some closely similar antigens. The findings show that the A/Eng antibody response with either vaccine is best in persons who already possess antibody to the A/HK strain. This response was also seen in the studies by Pereira *et al.* (1972) and Hoskins *et al.* (1973).

The large proportion of entrants with CF antibody against influenza A (65 %) is surprising even despite the occurrence of influenza A virus in the community just before and at the start of the trial. According to Hoyle & Fairbrother (1947) a CF antibody titre of 16 or higher is practically diagnostic of recent infection, while Fairbrother & Martin (1938) claim that such titres decline rapidly within six months. In the present study 266 (57 %) had a pre-vaccination titre of 16 and higher, and four months after vaccination there was little decline in titre. These findings suggest that the mere presence of CF antibody even at a titre thought to be significant cannot necessarily be taken as reliable evidence of a recent influenza infection.

The slight rise in CF antibody titres observed in those patients who received

the A vaccines (Fig. 2) was probably a response to vaccination rather than to infection since there was no comparable rise in CF antibody titre against influenza A in the control group.

The comparatively poor development of CF antibody with inactivated vaccine confirms the observation made by Hoyle & Fairbrother (1947) that the complement fixation test is not suitable to assess the antibody response to vaccination with influenza virus.

The routine influenza A virus reports from the Leicester Public Health Laboratory during the winter of 1972/73 were few in number and occurred almost entirely during November and December when the trial was starting. There was very little influenza diagnosed routinely during the follow-up period of the trial. The infrequency of clinical influenza was substantiated by the results of the trial in which only four of the 32 patients with influenza-like symptoms were shown to have been infected with influenza A virus. Of the remaining 28 patients, four yielded rhinovirus, respiratory syncytial virus and herpes simplex virus; some may have been due to influenza since their illnesses occurred in December and they showed an increase in antibody during the convalescent period, but this increase may also have been the result of vaccination. These findings underline the necessity for laboratory examination of suspected cases in trials of influenza vaccine. Had the present study been dependent on 'clinical' influenza the findings would have suggested that the vaccines did not protect, a conclusion which would have been unwarranted in view of the very few cases of proved influenza which occurred. Indeed a recent study (Hoskins *et al.* 1973) has clearly shown the efficacy of influenza vaccine containing the A/HK strain in protecting against A/Eng influenza.

It is of interest to note that in the control group 16 (10%) of the patients showed serological evidence of subclinical infection with influenza A virus as compared with only two overt cases. It would appear that at a time when influenza was apparently limited in Leicester the number of subclinical infections was proportionately much greater than the number of known cases. Miller *et al.* (1973) reported a survey in which about half of those persons with serological evidence of influenza A/HK infection during 1968/70 reported no illness. Our findings would indicate that subclinical infection is probably more prevalent than is generally recognized.

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