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A comparison of live and inactivated influenza A (H1N1) virus vaccines

1. Short-term immunity

Report to the Medical Research Council Committee on the Development of Vaccines and Immunization Procedures (Influenza Trials Sub-Committee)

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SUMMARY

Groups of volunteers were immunized subcutaneously with one of three inactivated influenza virus A/USSR/77 (H1N1) vaccine preparations; a whole virus vaccine, a surface-antigen subunit adsorbed vaccine, or an aqueous surface-antigen subunit vaccine. The reactions to immunization were recorded, and the antibody response was measured 1 month later. A fourth group of volunteers were inoculated intranasally with live attentuated A/USSR/77 (H1N1) influenza virus; the reactions and antibody response of these volunteers were also measured. One month after immunization, the incidence of infection by challenge with homologous live attentuated virus was determined for all groups of volunteers. The results showed that all four vaccines used were relatively non-reactogenic, and that inactivated vaccines induced higher titres of serum antibody than the live attenuated vaccine. All the vaccines induced significant protection against challenge virus infection which was directly related to the level of serum HI antibody response.

INTRODUCTION

A comparison of the problems associated with the production and acceptability of live and inactivated influenza virus vaccines suggests advantages for the former.

In addition, some studies have shown that live attenuated vaccines may induce a more solid immunity to challenge virus infection than inactivated vaccines. Such conclusions have been obtained in experimental animals (Potter, Jennings & McLaren, 1973) and in man (Beare *et al.* 1968; Freestone *et al.* 1972). We describe a study to compare the reactogenicity and protective efficacy of live attenuated and inactivated influenza A (H1N1) virus vaccines in student volunteers. The study compares three forms of inactivated influenza virus vaccine and a live attenuated vaccine. The incidence and nature of reactions to the vaccines were documented and antibody responses were measured using haemagglutination inhibition and neuraminidase inhibition tests. Protective efficacy was assessed by resistance to homologous attenuated challenge virus infection 1 month after immunization.

MATERIALS AND METHODS

Influenza virus vaccines

Three monovalent influenza virus A/USSR/77 (H1N1) vaccines, prepared by Glaxo Operations, Speke, were employed: an inactivated whole virus vaccine (WV) prepared by β-propiolactone inactivation of egg-grown virus purified by zonal centrifugation; a purified surface subunit vaccine adsorbed to Alhydrogel (AdSU) prepared by the methods of Brady & Furminger (1976); and a purified non-adsorbed aqueous surface antigen subunit vaccine (AqSU). All three vaccines were assayed for potency at the National Institute for Biological Standardization and Control, London, using the single radial diffusion techniques (Schild, Wood & Newman, 1975; Wood *et al.* 1977); in each case the potency employed was 9 μg of haemagglutinin per 0·5 ml inoculation volume.

Attenuated influenza virus clone 144-B (H1N1) was obtained from Dr B. K. Murphy, NIH, Bethesda, MD., U.S.A.: this virus vaccine is a recombinant of influenza A viruses ts/H2N2 and A/USSR/77 (H1N1), and has been shown to be attentuated in volunteers (Van Voorthuizen, Jens & Saes, 1981). The virus was supplied in ampoules containing $10^{7\cdot0}$ egg infectious doses (EID_{50}) per 0·5 ml, which was used to inoculate each volunteer intranasally.

Experimental design

Ninety-five students from the Universities of Sheffield and Birmingham and aged 18–20 years volunteered to take part in the study; all were in good health, and had no allergy to eggs. A blood sample was obtained from each volunteer and titrated for haemagglutination-inhibiting (HI) antibody to influenza virus A/USSR/77. On the basis of these results the volunteers were divided into five groups, each containing approximately the same number of individuals with no HI antibody and the same number with a similar geometric mean titre (gmt) of serum HI antibody. Each volunteer assigned to an inactivated vaccine group was immunized by the deep subcutaneous route with 0·5 ml of one of the three inactivated vaccines, or 0·5 ml of saline for the control group; a double blind design was employed. One month later each volunteer received a second dose of identical vaccine, when volunteers in the fifth group were inoculated intranasally with 0·5 ml of the attentuated influenza virus vaccine. One month later a blood specimen was obtained from each volunteer, who was then challenged intranasally with 0·5 ml

of attenuated virus vaccine; a third blood sample was obtained from each volunteer 1 month after this challenge-virus infection.

Reactions were assessed 24 h after both the first and second dose of inactivated vaccines, and categorized as mild local, local pain and systemic reactions and expressed as a percentage of the total number of questions (Jennings *et al.* 1978; Potter *et al.* 1980). Reactions were obtained relating to the attenuated virus vaccine each day for 7 days in terms of temperature and local and systemic symptoms, and expressed in the same way as for the inactivated vaccines. A temperature of $> 37{\cdot}5°$ was considered to be a febrile reaction.

Serological studies

Serum samples were coded and forwarded to the National Institute of Biological Standardization and Control for antibody estimations.

Haemagglutination-inhibiting (HI) antibody titres were estimated using standard techniques following treatment with cholera filtrate (Phillips-Duphar-B.V.) for 18 h at 37 °C followed by incubation at 56 °C for 1 h (W.H.O., 1953). Reference antisera were included in each assay.

Neuraminidase-inhibiting (NI) antibody titres were determined by the method of Aymard-Henry *et al.* (1973), using an influenza virus A(H1N1), derived by recombination of A/equine/Prague/56 (H1) and A/USSR/90/77 (N1) viruses, as the source of neuraminidase. For neutralization, the serum dilution – neuraminidase mixtures were held at 37 °C for 1 h, and the neuraminidase and fetuin substrate was incubated for 18 h at 37 °C.

RESULTS

Reactions to immunization

(A) *Reactions to inactivated virus vaccine*

The incidence of reactions to immunization with the three forms of inactivated influenza virus A/USSR/77 vaccine were assessed at 24 h. The incidence of the three types of reactions were calculated separately after the first and second doses and the results are shown in Fig. 1.

Volunteers given WV or AqSU showed a higher incidence of local reactions after the second dose (23·5 and 22·9%) compared to the first dose (11·8 and 7·9%). The incidence of local reactions to AdSU vaccine was higher than for the other preparations after both the first and second dose (37·5% and 38·1%). In all cases these reactions were reported as mild, and did not persist beyond 48 h. The incidence of local pain was considerably higher after both doses of AdSU vaccine compared to the WV and AqSU vaccines (Fig. 1). However, reactions were mild in nature and of short duration. No systemic reactions were reported following immunization with WV or AqSU vaccines, but a few mild systemic reactions were reported following inoculation with AdSU vaccine. Thus, all three types of reaction were more common following immunization with AdSU vaccine than following immunization with WV or AqSU vaccines.

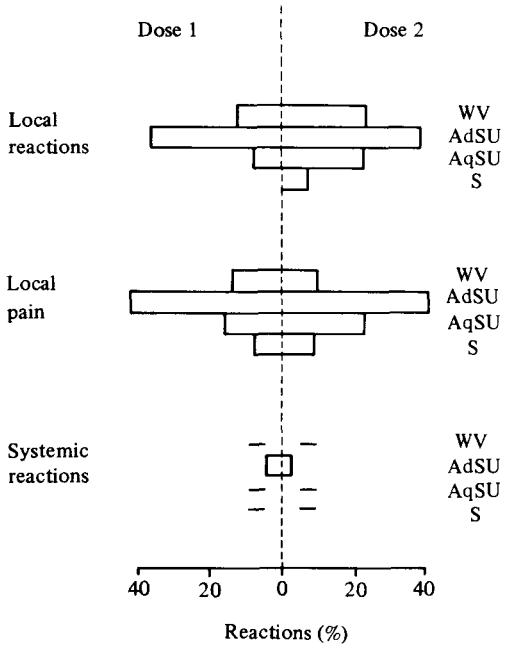

Fig. 1. Incidence of reactions to immunization with inactivated influenza-virus vaccine. WV, Whole virus; AdSU, Subunit adsorbed; AqSU, aqueous subunit; S, saline.

(B) *Reactions to attenuated virus vaccine*

The incidence of local and systemic reactions following the inoculation of volunteers with live attenuated virus vaccine is shown in Fig. 2. Prior to inoculation (day 0), the incidence of local symptoms was 8·0 % with no systemic symptoms. Following inoculation, the incidence of local reactions increased to 15·8 % on day 1, 10·8 % on day 2 and 9·2 % on day 3; after this time, the incidence was lower than on day 0 (Fig. 2). The incidence of systemic reactions was 1·7 % on the first and 0·8 % on the second day after inoculation, and none were reported after that time. No febrile reactions were observed. Both local and systemic reactions were mild; thus, the live vaccine used in this study was adequately attenuated, producing neither febrile reactions, nor significant symptoms.

Serum HI antibody response to immunization

Serum HI antibody levels prior to the first and 28 days after the second dose of inactivated virus vaccines are shown in Table 1. Of the 18 volunteers given WV, 6 had HI titres of > 30 prior to immunization, compared to 15 following immunization. Similar increases in serum HI antibody titre were seen following immunization with AdSU and AqSU vaccines. Of the 23 volunteers given live attenuated virus vaccine, 2 had antibody titres of > 30 prior to immunization, compared to 12 following immunization (Table 1). No increase in HI antibody titre were observed following immunization with the control saline; thus, there was no evidence of natural infection by influenza virus A (H1N1) in the study populations during the period of observation.

The aggregate increases in serum HI antibody titre measured in the five groups

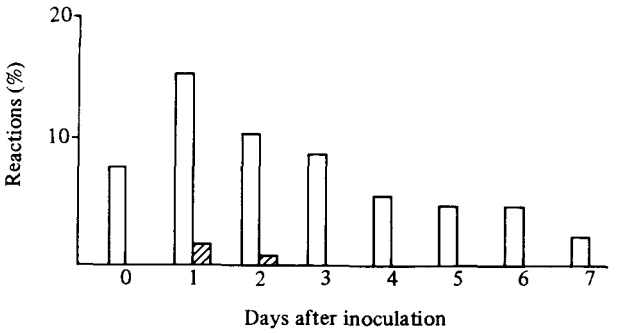

Fig. 2. Incidence of reactions in volunteers inoculated with live attenuated influenza virus. □, Local reactions; ▨, systematic reactions.

Table 1. *Serum HI antibody response of volunteers to immunization with influenza A/USSR/77 (H_1N_1) virus vaccines*

Vaccine given	No. of volunteers	Serum specimen	Serum HI antibody response to A/USSR/77 virus					
			< 10	10–20	30–40	60–120	160–480	≥480
WV	18	Pre-imm.	7	5	2	2	2	—
		Post-imm.	3	—	1	4	8	2
AqSU	16	Pre-imm.	11	1	2	2	—	—
		Post-imm.	5	1	2	2	2	3
AdSU	15	Pre-imm.	10	3	—	1	1	—
		Post-imm.	1	—	1	6	3	4
Saline control	23	Pre-imm.	13	6	2	1	1	—
		Post-imm.	13	6	3	—	1	—
Live	23	Pre-imm.	18	3	—	1	1	—
		Post-imm.	8	3	3	8	1	—

WV denotes whole inactivated virus; AqSU, aqueous subunit vaccine and AdSU, adsorbed subunit vaccine.

of volunteers are shown in Table 2. The 50 % protective level of serum HI antibody against challenge-virus infection has been calculated as 30–40 (Meiklejohn *et al.* 1952; Hobson, Beare & Ward-Gardner, 1972; Potter & Oxford, 1979); the results, therefore, were also analysed to give the percentage incidence of HI antibody titres of ⩾ 40. Of the 18 volunteers given WV vaccine, 14 (78 %) showed a 4-fold rise in HI antibody titre with a gmt increase from 17·6 to 109·4 and 78 % had titres of ⩾ 40. Of the fifteen volunteers given AdSU vaccine, 14 (93 %) showed a 4-fold rise in serum HI antibody titre with a gmt increased from 9·5 to 177·7, and 13 (87 %) had HI titres of ⩾ 40. In contrast, only 9 (56 %) of the 16 volunteers given AqSU vaccine showed a 4-fold rise in serum HI antibody titre and a gmt rise from 9·7 to 48·1 with 7 (44 %) producing HI antibody titres of ⩾ 40. The results show that the greatest serum HI antibody response to immunization was seen in volunteers given AdSU vaccine, and the least response was found in those given AqSU vaccine: this was the reverse order for reactogenicity to vaccines, where the AdSU vaccine was reported to be the most reactogenic and the AqSU the least (Fig. 1). Of the volunteers given live attenuated virus vaccine, 12 (52 %) were infected as

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Table 2. *Response of volunteers to immunization and subsequent challenge with homologous attenuated influenza A/USSR/77 (H_1N_1) virus*

Vaccine given*	No. of volunteers	Response to immunization: No. rises of ⩾ 4-fold No. (%)	Response to immunization: No. titres ⩾ 40 No. (%)	Response to immunization: Change in gmt	No. infections following challenge No. (%)
WV	18	14 (78)	14 (78)	17·6–109·4	2 (11)
AqSU	16	9 (56)	7 (44)	9·7–48·1	3 (19)
AdSU	15	14 (93)	13 (87)	9·5–177·7	2 (13)
Saline control	23	—	1 (4)	10·4–9·3	16 (70)
Live	23	12 (52)	9 (39)	7·5–21·4	7 (30)

* For abbreviations see Table 1.

shown by a 4-fold rise in serum HI antibody titre, the gmt increased from 7·5 to 21·4 and 9 (39%) had HI antibody titres of ⩾ 40.

Neuraminidase-inhibition (N1) antibody titres

Following immunization with inactivated WV, AqSU and AdSU the gmt of serum NI antibody increased from 2·3 to 3·2, 2·0 to 2·9 and 2·1 to 3·8, respectively. The live vaccine induced a change in gmt from 2·4 to 2·7 with no evidence of natural infection in the control group (gmt 2·2–2·3). Thus, none of the vaccines induced a significant increase in serum NI antibody titre.

Incidence of infection following challenge-virus inoculation

In order to compare the incidence of infection by the challenge virus given 1 month after immunization with either attenuated or inactivated virus vaccine, the volunteers were considered in five groups: those who produced a ⩾ 4-fold increase in serum HI antibody titre following immunization with inactivated virus vaccine (inactivated vaccine responders); volunteers who failed to produce a ⩾ 4-fold HI antibody response to inactivated vaccine (inactivated vaccine-non-responders); volunteers infected with live virus vaccine, as shown by a ⩾ 4-fold increase in serum HI antibody titre (live vaccine: infected); volunteers who did not produce a ⩾ 4-fold increase in serum HI antibody titre following inoculation of attenuated virus vaccine (live vaccine: non-infected); and the control volunteers given saline. There was no significant difference between the three inactivated vaccines in the proportion of infections following live attenuated challenge based on analysis using a chi-squared test for a difference in the proportions ($\chi^2 = 0{\cdot}41$, D.F. $= 2$, $P > 0{\cdot}1$). Therefore, the three groups of volunteers given inactivated vaccines were analysed as a single group. The proportion of infections amongst the three basic groups, inactivated vaccine (7/49, 14%) live vaccine (7/23, 30%) and controls (16/23, 70%) were compared. A multiple comparison procedure for examining the contrasts between the three pairs showed significant differences ($P < 0{\cdot}01$) between both the inactivated vaccine and the controls and the live vaccine and the controls. However, the difference between the live and inactivated vaccines was not significant ($P > 0{\cdot}1$).

Table 3. *Immunity to challenge-virus infection in relation to the antibody response following immunization with different types of vaccine*

Vaccine given	No. of volunteers	Serum HI antibody titres for influenza A/USSR/77 virus						Total infections (%)	
		< 10	10–20	30–40	60–120	160–480	⩾ 480		
Inactivated (responders)	37	—	1/1*	1/4	1/12	0/11	0/9	3 (8)	7 (14)
Inactivated (non-responders)	12	3/9	1/1	—	—	0/2	—	4 (33)	
Live (infected)	12	-	0/2	0/3	0/6	0/1	—	0 (–)	7 (30)
Live (non-infected)	11	5/8	1/1	—	1/1	0/1	—	7 (64)	
Saline only	23	9/13	6/6	1/3	—	0/1	—	16 (70)	
Total (Percentage)	95	17/30 (57)	9/11 (82)	2/10 (20)	2/19 (11)	0/16 (—)	0/9 (—)	30 (32)	

* Number infected with challenge/number of volunteers.

The total number of infections by challenge virus was 32%, and the incidence was related to the serum HI antibody response following immunization (Table 3). Three of 37 volunteers (8%) who responded to inactivated vaccine became infected with the challenge virus, but four of the 12 (33%) who did not respond to inactivated vaccine became infected. None of the twelve volunteers who were infected with the live attenuated vaccine were infected by the subsequent live virus challenge. However, seven of the 11 (64%) volunteers who did not respond to the live virus vaccine and 16 of the 23 (70%) volunteers who were given saline became infected when challenged with live virus. Analysis using a chi-squared test for differences in proportions found that the differences between the live and inactivated vaccines in the proportion of antibody responder that were infected with the challenge virus were not significant ($P > 0{\cdot}1$), and the differences in the proportion of non-responders that were infected with challenge virus were not significant either ($P < 0{\cdot}1$). However, there was a highly significant difference between the proportion of responders that became infected and the proportion of non-responders that became infected with the challenge virus ($P < 0{\cdot}01$). Furthermore, the proportion of antibody responders in the group given inactivated vaccine was just significantly ($P \simeq 0{\cdot}05$) greater than in the group given live vaccine.

DISCUSSION

The results of previous comparative studies indicated that an attenuated influenza A virus vaccine induced lower serum HI antibody responses, but an apparently higher level of immunity to challenge-virus infection compared to that observed following immunization with an inactivated vaccine. The conclusion from this and another similar study was that attenuated virus vaccines have this advantage (Beare *et al.* 1968; Freestone *et al.* 1972). This study used an influenza

virus A/USSR/77 vaccine. Successful infection with a live attenuated influenza A (H1N1) virus was achieved in approximately half the volunteers following one dose, and only a few mild reactions to immunization were reported. The attenuated vaccine induced a relatively low serum HI antibody response, but good immunity to challenge-virus infection. In contrast, immunization with inactivated vaccines produced some reactions, though these were mild in nature and of short duration. The inactivated vaccines produced relatively high serum HI antibody titres with 75% seroconversions and induced immunity to challenge-virus infection. A single dose of live virus vaccine did not induce as many seroconversions as the inactivated vaccines. In the present study, two doses of inactivated vaccine caused a $\geqslant$ 4-fold rise in HI antibody in 75% of volunteers, whilst a single dose of attenuated vaccine induced an increase in 52% of volunteers, and two doses (the one given as vaccine plus the one given as a challenge) induced a $\geqslant$ 4-fold rise in 83% of volunteers. Thus, two doses of attenuated vaccine induced slightly more seroconversions than two doses of inactivated vaccine, although the total titres of antibody were higher for volunteers given the latter vaccine. In all the groups considered, the incidence of challenge-virus infection was inversely related to the serum HI antibody response to immunization.

The analysis of the results indicates that immunity to challenge infection was related to antibody response following immunization. Although there is no significant evidence in the data that immunity to challenge infection is other than the same for both live and inactivated vaccine groups, the inactivated vaccine induced significantly more seroconversions and as a result probably confers greater immunity to challenge virus infection significantly more often, as a large study might reveal.

The acceptability of a vaccine to the public depends upon many factors, including the requirement for injection and reactions to the preparation. Thus, live attenuated virus vaccines have advantages which must be contrasted to the slightly more reactogenic inactivated vaccines that induce a better antibody response which is associated with more solid immunity.

The role of systemic antibody must be contrasted to local antibody formation, as in studies of influenza virus vaccines in ferrets where infection with live virus was shown to induce lower serum HI antibody titres, but a more solid immunity to challenge virus infection than inactivated vaccines (Potter, Jennings & McLaren, 1973). Virus infection was found to induce local neutralizing antibody in ferrets while inactivated viruses did not; and this could be the reason for the more solid immunity associated with live virus vaccines in the ferret model. In human volunteer studies higher titres of local neutralizing antibody have been demonstrated following infection with attenuated virus vaccines than following immunization with inactivated vaccines, and the more solid immunity associated with live attenuated vaccines may be due to their ability to stimulate better local antibody production (Waldman *et al.* 1968; Kasel *et al.* 1969). However, the live attenuated virus vaccine employed in this study did not induce significantly better immunity to challenge-virus infection compared to the inactivated vaccines; moreover, the local antibody response to immunization has been shown to be short-lived (Murphy *et al.* 1973; Ruben, Potter & Stuart-Harris, 1975). In the present study, and in the previous studies (Beare *et al.* 1968; Freestone *et al.* 1972), challenge-virus infection was given 1 month after immunization; it remains to be determined if

the differences in immunity for volunteers given live or inactivated virus vaccines are demonstrable over a longer period.

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