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Comparison of the protective efficacies of the live vaccine RIT 4025 and an inactivated vaccine against a natural heterologous A/Victoria/3/75 infection

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SUMMARY

A clinical trial was initiated in South Africa before the winter season of 1976. The study involved 253 volunteers divided into three groups of vaccinees and one control group.

Two groups of vaccinees were inoculated with either one or two doses at 2 weeks' interval ($10^{7.2}$ EID 50/dose) of the RIT 4025 live recombinant strain [A/Scotland/840/74 (H3N2) serotype] and one group received one injection of an inactivated vaccine [A/Port Chalmers/1/73 (H3N2), 360 i.u., A/Scotland/840/74 (H3N2), 300 i.u. and B/Hong Kong/8/73, 300 i.u./dose].

The serum antihaemagglutinin antibody responses against the heterologous A/Victoria/3/75 strain as measured by the single radial haemolysis test were satisfactory and not statistically different in all groups of vaccinees. On the other hand, the antineuraminidase antibody response was better in the group receiving the killed vaccine.

At the end of the influenza season, A/Victoria/3/75 infections were confirmed serologically.

Only 12% of the infections were symptomatic. The infection rate was significantly reduced in the live vaccine groups, whereas in the killed vaccine group the percentage of infection was lower but not significantly different from that in the placebo group.

INTRODUCTION

Comparison of the protective efficacies of live and killed influenza vaccines has been performed by artificial challenge or by surveillance during epidemics.

Challenge studies have demonstrated that live vaccines induced a better protection than did the usual killed vaccines (Beare *et al.* 1968; Freestone *et al.* 1972; Hobson *et al.* 1973).

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Table 1. *Vaccination schedule*

	Day 0 (29/3/76)	Day 14 (12/4/76)
Group N 1 dose live vaccine	1 dose live vaccine IN 1 dose placebo s.c.	1 dose placebo IN 1 dose placebo s.c.
Group NN 2 doses live vaccine	1 dose live vaccine IN 1 dose placebo s.c.	1 dose live vaccine IN 1 dose placebo s.c.
Group KV 1 dose killed vaccine	1 dose placebo IN 1 dose killed vaccine s.c.	1 dose placebo IN 1 dose placebo s.c.
Group P Placebo controls	1 dose placebo IN 1 dose placebo s.c.	1 dose placebo IN 1 dose placebo s.c.

The lyophilized live vaccine was administered by drops intranasally in a 0·5 ml volume to the subjects in supine position with the head hyperextended.

The virus concentration of the vaccine was $10^{7\cdot2}$ EID 50/dose. The killed vaccine contained A/Port Chalmers/1/73 300 i.u., A/Scotland/840/74 300 i.u., and B/Hong Kong/8/73 300 i.u., delta-aluminium oxide, 2·4 mg as adsorbant and Thiomersal 50 μg per dose. It was distributed by Noristan Laboratories. It was administered subcutaneously in a dose volume of 0·5 ml. The placebos corresponding to each vaccine did not contain any virus or antigens. The placebo for intranasal use (IN, Table 1) was lyophilized and reconstituted as the vaccine before administration. The placebo for injection (s.c., Table 1) was isotonic saline.

Study procedure and population

The vaccination trial started on 29 March 1976. It involved a total of 253 healthy white students staying at the University Hostel in Pretoria.

Volunteers having acute respiratory or febrile illnesses were excluded from the study. Also excluded were patients treated with immunosuppressive drugs or corticosteroids (more than 10 mg prednisone/day), and volunteers allergic to neomycin or chicken proteins. Furthermore, accepted volunteers had not received any influenza vaccine within 6 months before the start of the trial.

Vaccination schedule

This is shown in Table 1. Four groups of randomly allocated volunteers received under double blind conditions, one dose of live vaccine (N), two doses of live vaccine (NN) at 2 weeks' interval, one dose of killed vaccine (KV) or placebo (P).

Epidemic survey

At the time of vaccination each volunteer received a card to be completed with the assistance of a medical student in the hostel each time an upper respiratory illness or fever occurred.

Blood samples were collected before the first vaccination, 21 days after the second vaccination and at the end of the influenza season from most of the volunteers. A convalescent blood sample was also taken from those who complained of clinical symptoms.

Table 2. *Significant antihaemagglutinin antibody rises against A/Victoria/3/75 after vaccination or administration of a placebo preparation*

Vaccine group (number)	SRH before vaccination ⩽ 22 mm²	⩽ 50 mm²	Total
Group P (n = 48)	0/34 (0)*	0/39 (0)	0/48 (0)
Group N (n = 40)	14/18 (78)	18/25 (72)	24/40 (60)
Group NN (n = 46)	20/26 (77)	24/32 (75)	29/46 (63)
Group KV (n = 48)	23/27 (85)	32/38 (84)	35/48 (73)

* Number of significant antibody rise/total number with indicated prevaccinal SRH value; ( ) = %.

Table 3. *Cumulative percentages of SRHH antibody levels and mean SRHH titres against A/Victoria/3/75 virus before and after administration of vaccines or placebo*

Vaccine group		Cumulative percentages of titres (mm²) against A/Victoria/3/75 ⩾ 20	⩾ 40	⩾ 60	⩾ 80	Mean titres (mm²)
P	Pre	40	23	15	6	23
	Post	40	23	15	6	23
N	Pre	62	55	22	5	⩾ 36·4
	Post	93	87	60	17	⩾ 62
NN	Pre	52	37	26	9	31
	Post	83	83	63	26	⩾ 60
KV	Pre	49	30	15	4	26
	Post	92	81	64	33	⩾ 68

The percentages of antibody rises against the Victoria strain were not significantly different for the groups receiving one or two doses of the live vaccine or one dose of the killed vaccine (χ^2 test). No response occurred in the placebo group.

Table 3 shows the cumulative percentages of volunteers in each group having an SRHH value above 20, 40, 60 and 80 mm², before and after vaccination, as well as the mean titres. The post-vaccination distribution of titres is similar for the three groups of vaccinees.

The mean titres could not be exactly determined since for some sera the outlines of haemolytic zones merged (diameter greater than 11 or 12 mm); however, they were greater than 60 mm² in all groups of vaccinees. Titres before vaccination were on average higher in group N. In fact, the percentage of sera having titres from 3·9 to 40 mm ² before vaccination was significantly higher than the corresponding percentage in the placebo group (χ^2, $P < 0{\cdot}01$). There was no significant difference in the prevacination distribution of titres between the other two groups and the placebo group. The difference in group N can be attributed to chance.

Antineuraminidase antibodies were also measured by the SRHH method using X48 (Heq1N2), an antigenic hybrid which has the neuraminidase of the Victoria strain.

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Table 4. *Significant antineuraminidase antibody response in vaccinees who had a significant rise in SRH/H*

Group	Proportion of significant responses *
NN	11/29 (39%) †
N	9/25 (36%) †
KV	23/33 (70%) ‡
Natural infection	28/34 (82%)

* Only in subjects with antibody level ⩽ 50 mm² before vaccination.
† χ^2 square with Yate's correction: $0{\cdot}05 < P < 0{\cdot}025$.
‡ χ^2 square with Yate's correction: $P < 0{\cdot}001$.

Table 5. *Incidence of A/Victoria/3/75 natural infections*

Group	Number tested	Number infected (%) (significant SRHH antibody rise)	Number with flu symptoms	Flu illnesses serologically confirmed (%)
P	48	17 (35) *	6	2 (4·2)
N	40	5 (12·5) *	5	2 (5)
NN	46	4 (8·7) †	4	0 (0)
KV	48	8 (16·7)	8	0 (0)
Total	182	34 (18·7)	23 (12·6)	4 (2·2)

* χ^2 square with Yate's correction: $0{\cdot}025 < P < 0{\cdot}05$.
† χ^2 square with Yate's correction: $0{\cdot}001 < P < 0{\cdot}01$.

Table 4 shows the percentages of significant antibody rises in each group for those volunteers having SRHN values below 50 mm² before vaccination and who had responded against the haemagglutinin (no responses against neuraminidase were detected in volunteers who had not responded against the haemagglutinin).

The killed vaccine induced a significantly higher percentage of antineuraminidase antibodies than the live vaccine. Similar results were obtained (not shown in the table) when the X42 (Heq1N2) hybrid was used (neuraminidase of the A/Port Chalmers/1/73 strain). For comparison, natural A/Victoria/3/75 infections induced antineuraminidase antibodies in 28/34 (82%) volunteers who had responded to the haemagglutinin after the winter period.

Surveillance programme

The total incidence of natural infection among participants who completed the study, as detected by a significant increase in the third blood sample, is 18·7% (Table 5). Analysis of findings in the different groups showed that an infection rate of as high as 35% was observed in the placebo group although only two subjects (4%) reported clinical influenza. Two volunteers who had low antibody titres before vaccination and had received one dose of the live vaccine also reported symptoms. They had not seroconverted after vaccination. These four

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clinical cases occurred in July 1976, which therefore seems to have been the approximate date of occurrence of this mild outbreak.

Nineteen out of 23 flu-like illnesses (Table 5) have to be attributed to upper respiratory illnesses other than influenza A or B, since they did not seroconvert for the A/Victoria/3/75 or B/Hong Kong/8/73 (not shown in the tables) strains. The haemagglutination inhibition technique (HI) was used to titrate against B/Hong Kong/8/73. The difference in infection rates between the placebo and the NN groups is significant (Table 5). Differences between the placebo and killed vaccine groups and between the groups of vaccinees are not significant (Table 5).

In group N, the rate of infection is also significantly lower than in the group P but this protection may partly be due to naturally acquired antibodies since these volunteers had higher prevaccination titres (Table 3). The protection rates against infection are 75%, 64% and 53% for the NN group, N and KV groups respectively.

The incidence of illness in the present study was too small to calculate protection against illness.

DISCUSSION

The single radial haemolysis (SRH) test was chosen for the study of the serological response in a vaccination trial comparing the live recombinant strain RIT 4025 (A/Scotland/840/74) administered once or twice and a killed vaccine containing a mixture of A/Scotland/840/74 and A/Port Chalmers/1/73 antigens.

The SRH test is more rapid and detects a higher number of natural infections than does the HI test (Delem & Jovanovic, 1978). Since the epidemic strain in South Africa during the winter season 1976 was A/Victoria/3/75, antihaemagglutinin antibody rises after vaccination were evaluated against the heterologous A/Victoria/3/75 strain. The response was satisfactory and similar in all groups of vaccinees. There was a higher antineuraminidase antibody response in the killed vaccine group and after natural infection than after live vaccine. A possible explanation for this finding is that the period of replication of the vaccine virus may be too short to allow the expression of the antigenicity of the neuraminidase. These results are similar to those obtained by Rubin *et al.* (1976) for the 'Alice' strain using the enzymatic titration method of WHO (Aymard-Henry & Coleman, 1973). However, little attention is usually paid to antineuraminidase antibody responses since the antihaemagglutinin antibody level is the most important factor to evaluate the degree of protection (for references see Delem & Jovanovic, 1978).

Surprisingly, most of the subjects who were infected as detected by the SRH test after the influenza season had no symptoms. Among the 34 volunteers infected, only four reported clinical symptoms. This could be due to a change of the virulence of the A/Victoria/3/75 strain or to a low susceptibility of the population. Some variation in intrinsic viral virulence does probably exist, explaining, for instance, the severity of an H1N1 epidemic in Liverpool in 1954 (Stuart-Harris, 1973) compared with the mild pandemic of 1947 (Langmuir, Henderson & Serfling,

1964). However, as explained by Kilbourne (1975) the circulation of the virus in the community, in the form of subclinical infections, is probably what allows the persistence and spreading of the virus.

In our trial a high level of immunity was reached in the population after vaccination since 75% of participants were immunized, thus probably producing some herd immunity. As a result only the protection rate against infection could be evaluated. The results showed that two doses of the live RIT 4025 vaccine gave 75% protection whereas the killed vaccine conferred 54% protection.

This observation reinforces data obtained in other studies which showed a trend towards better protection against natural infection after immunization with live vaccines.

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