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SECTION 3

RECENT ADVANCES IN THE DESIGN AND EVALUATION OF INACTIVATED WHOLE PARTICLE AND SUBUNIT VACCINES

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Placebo-controlled double-blind clinical studies on the efficacy of different influenza vaccines assessed by experimental and natural infection

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Summary

Four placebo-controlled double-blind studies on the protective efficacies of a freeze-dried aerosol and an injectable whole-virion inactivated influenza vaccine, each containing 400 i.u. A/Port Chalmers/1/73 (H_3N_2) and 240 i.u. B/Hong Kong/8/73 per dose, were carried out on a total of 601 subjects using three different live influenza vaccines as challenge virus. In the second of these studies a tween-ether 'split' aluminium-absorbed injectable vaccine containing 400 CCA units A/Port Chalmers/1/73 (H_3N_2) and 300 CCA (chick cell agglutination) units B/Hong Kong/8/73 was also tested. Challenge in the first three studies occurred 3 weeks after vaccination whereas in the last study it took place 3 months after vaccination. The live vaccines were recommended for the 1974–75 season in Belgium, Rumania and Yugoslavia in which countries the studies were performed and contained an A/England/42/72 (H_3N_2)-like strain, a B/Victoria/98926/70-like strain and an A/Port Chalmers/1/73 (H_3N_2)-like strain respectively. The latter vaccine was used in both of the last two studies. Infection with the vaccine strain was diagnosed by virus isolation and/or serological response after challenge since this produced negligible clinical signs and symptoms.

The aerosol vaccine showed infection protection rates of 59% ($P = 0{\cdot}0075$), 42% ($P < 0{\cdot}005$), 26% ($P = 0{\cdot}47$) and 36% ($P = 0{\cdot}19$) and the whole-virion vaccine rates of 84% ($P < 0{\cdot}005$), 25% ($P = 0{\cdot}025$), 80% ($P = 0{\cdot}09$) and 88% ($P = 0{\cdot}01$) respectively. The tween-ether 'split' vaccine included in the second study gave 21% ($P = 0{\cdot}06$) protection against a very heterologous type-B virus.

It is argued that results in such studies are biased in favour of injectable vaccines when infection is diagnosed by serology alone whereas the bias is in favour of an aerosol vaccine if this is done by virus isolation alone. When challenge was with the type-A vaccines most 'takes' were diagnosed only on the basis of a serological response. With these two vaccines an inverse relationship existed between pre-challenge serum HI

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(haemagglutination-inhibiting) antibody levels against the challenge strain and 'take' rate. With the type-B vaccine, on the other hand, virus isolation commonly occurred in the absence of sero-conversion and there was no correlation between level of serum HI antibodies and 'take' rate.

In a placebo-controlled double-blind field trial conducted in parallel on 1326 subjects in the same population as the last challenge study, the aerosol vaccine gave 63% ($P = 0{\cdot}09$) and the whole-virion vaccine only 35% ($P = 0{\cdot}37$) protection against serologically confirmed influenza.

It is concluded that challenge studies using a live vaccine as challenge virus can yield statistically significant results and that the efficacy of inactivated vaccines can be validly compared if they are administered by the same route. Such studies can be conveniently conducted on large numbers of subjects and this method of assessing vaccine efficacy deserves to be further evaluated.

Introduction

The value of any influenza vaccine should be assessed by measuring the protection against infection and disease which it confers to subjects who would normally be vaccinated in routine practice. Influenza vaccine field trials are, however, prone to give inconclusive results (Hobson, 1975). Factors which contribute to this unsatisfactory state of affairs are the difficulty of obtaining a strictly comparable unvaccinated control group (preferably placebo vaccinated), the unreliability of the clinical diagnosis of influenza, the continual mutation of the causative viruses and the notorious reluctance of influenza to strike where vaccine trials have been set up. Thus, attempting to assess the protective efficacies of different influenza vaccines under field conditions can, and often does, prove to be frustrating.

Moreover, for logistic reasons, it is usual to test in field trials only one vaccine at a time but, owing to the many variables which affect the outcome of such studies, it is not appropriate to compare the relative merits of different vaccines when these have been tested separately.

Some workers have conducted informative challenge studies on limited numbers of healthy volunteers by using partly attenuated viruses to assess vaccine-induced immunity (Freestone *et al.*, 1972; Hobson *et al.*, 1972; Couch, 1975).

However, such studies pose ethical problems and must usually be performed in closed communities to reduce further the small risk of virus spread. A convenient and generally accepted method of simultaneously evaluating different vaccines in open communities is still much needed.

The availability and general use of highly attenuated live influenza vaccines in several countries offers the opportunity to challenge at will large numbers of subjects. Thus, if comparable groups of potential vaccinees are administered either vaccines or placebo some time before receiving the live vaccine, the protective efficacies of different vaccine could be simultaneously assessed by measuring the 'take' rate of the attenuated virus in the various groups. Such investigations can be conducted in open communities and satisfactorily controlled. Infection can be conveniently confirmed in the laboratory since exposure to a known virus occurs at a predetermined time. Furthermore, few ethical objections can be raised against such studies.

In this paper some of the findings in four separate studies using this method of assessing vaccine efficacy will be described. One of these challenge studies was performed on randomly selected subjects from a conventional field trial with the object of comparing the protection rates of two types of vaccines as assessed by both experimental and natural infection.

Materials and methods

Study populations and live vaccines

In Table 1 are recorded the location of the study centre, the live vaccine used as challenge virus and the number of subjects involved in each study. In Belgium and Rumania, the virus strain in the live vaccine available for general use during the 1974–75 season was antigenically different from that in the inactivated vaccines under test but in Yugoslavia the strain in the live and inactivated vaccines were homologous. Subjects in Rumania and Yugoslavia were college students and in Belgium they were inmates of a psychiatric hospital.

Inactivated vaccines

The type, route of administration and antigenic composition of the inactivated vaccines used in the various investigations are shown in Table 2. In the subsequent tables these three types of vaccines will be referred to as 'Influvac Plain', 'Influvac Spray' and 'Cantacuzino Institute' respectively. 'Influvac Plain' and 'Cantacuzino Institute' vaccines were administered with syringe and needle and 'Influvac Spray' as previously described (Liem *et al.*, 1973).

Study procedure

The procedure for the challenge studies was basically the same in all centres. After blood samples had been obtained, subjects were randomly allocated to different groups and received either placebo or one dose of one of the inactivated vaccines. In order to keep the studies double-blind both spray and injectable placebos were used but these two sub-groups of subjects were combined for statistical analysis. In the challenge study forming part of the field trial no pre-study blood samples were obtained. Three weeks

TABLE 1. Study centre, challenge virus and number of subjects for each study

Study	Study centre	Dose and strain of challenge virus	Number of subjects
Challenge study I	St Niklaas, Belgium	$10^{7\cdot2}$ EID_{50} A/Eng/42/72-like (R.I.T. vaccine)	122
Challenge study II	Bucharest, Rumania	$10^{6\cdot7}$ EID_{50} B/Vic/98926/70-like (Russian vaccine)	274
Challenge study III	Zagreb, Yugoslavia	$10^{4\cdot4}$ EID_{50} A/PC/1/73-like (Yugoslav vaccine)	81
Challenge study IV	Zagreb, Yugoslavia	$10^{4\cdot8}$ EID_{50} A/PC/1/73-like (Yugoslav vaccine)	124
Field trial	Zagreb, Yugoslavia	? (Natural exposure) ? A/PC/1/73-like (Wild A virus)	1326

TABLE 2. Type, route of administration and antigenic composition of tested inactivated influenza vaccines

Vaccine type	Administration route	Composition per dose	Name of vaccine
Whole-virion, aqueous	Subcutaneous	A/PC/1/73—400 i.u. B/HK/8/73—240 i.u.	'Influvac Plain'
Whole-virion, freeze-dried	Intranasal	A/PC/1/73—400 i.u. B/HK/8/73—240 i.u.	'Influvac Spray'
'Split' Al-absorbed	Intramuscular	A/PC/1/73—400 CCA u. B/HK/8/73—300 CCA u.	'Cantacuzino Institute'

TABLE 3. Specimens collected and virus isolation techniques in different study centres

Study centre	Specimens for virus isolation	Collection days (post-challenge)	Isolation technique
St Niklaas, Belgium	Nasal washings and throat swabs	2, 3, 4	Allantoic inoculation
Bucharest, Rumania	Throat swabs	1, 2, 3	Allantoic inoculation + Amniotic passage
Zagreb, Yugoslavia	Nasal washings and throat swabs	2, 3	Allantoic inoculation

after vaccination a second blood sample was obtained and subjects were administered one dose of live vaccine by the technique recommended by the respective vaccine manufacturers. In Belgium and Rumania inoculation was by nasal drops but in Yugoslavia this was by a fine nasal spray. Challenge doses (in EID_{50}), as determined after reconstitution of the freeze-dried vaccines, were $10^{7\cdot2}$, $10^{6\cdot7}$, $10^{4\cdot4}$ and $10^{4\cdot8}$ in the four studies respectively (Table 1).

Challenge in the last study was 3 months after vaccination and, as it happened, this was only a few days before a small influenza outbreak occurred in the study centre which was the one where a field trial had also been set up. For at least 5 days after challenge, subjects were clinically examined and any sign or symptom recorded on individual subject forms.

Virus isolation

Specimens for virus isolation were obtained as indicated in Table 3. All specimens were processed within 24 hr in the nearest laboratory. In Rumania, one amniotic passage was carried out after primary allantoic inoculation of throat specimens in 10-day-old chick embryos and all embryonic fluids were titrated for haemagglutinin with both rooster and guinea-pig erythrocytes after 72 hr of incubation. In the other centres, only primary allantoic inoculations and rooster erythrocytes were used for detection

of haemagglutinating virus in clinical specimens. Any isolated haemagglutinating virus was assumed to be the challenge strain.

Serum antibody titrations

In Belgium and Rumania a final blood sample was obtained 3 weeks after challenge. In the two Yugoslav studies the final blood samples were collected 4 and 5 weeks after challenge respectively. All sera from the same subject were kept frozen until simultaneously titrated by a standard microtitre technique for HI antibodies against the challenge strain or a virus homologous to it. Cholera filtrate (Philips-Duphar B.V., Amsterdam) was used to inactivate non-specific inhibitors.

In the field trial those who fell ill had acute and convalescent sera collected and these were titrated for HI antibodies against A/Port Chalmers/1/73 (H_3N_2), A/Scotland/840/74 (H_3N_2) and B/Hong Kong/8/73. All titrations were performed in the same laboratory.

Results

Comparability of groups

In all studies the groups were found to be comparable with regard to the relevant parameters of age, sex and pre-vaccination serum HI tires. The pre-vaccination serum HI titres were, however, not determined in the fourth study.

Clinical response to challenge

The live vaccines produced no symptoms in the great majority of subjects. Four subjects in the second Yugoslav study—three in the placebo and one in the spray vaccine group—had mild to moderate clinical influenza confirmed by serology but this could have been caused by the wild type-A virus concurrently circulating in the study centre. The only commonly observed sign was an injection of the throat and soft palate around the uvula. This was present in nearly all subjects challenged with the type-A vaccines but in just over 50% of those receiving the type-B vaccine.

Diagnosis of infection

The clinical signs and symptoms were very mild and not diagnostic of infection. 'Take' of the live vaccines had therefore to be assessed on laboratory findings alone, and is defined as at least one virus isolation or a four-fold or greater rise in serum HI titre after challenge or both.

First study

Table 4 records the findings in the first study on those subjects who completed the study. Both 'Influvac Spray' and 'Influvac Plain' gave statistically significant protection compared to placebo. The difference in the 'take' rates in the two vaccine groups is not significant. It is noteworthy that the 'take' rate in the spray vaccine group was dictated by serology alone whereas both virus isolation and serology were of diagnostic importance in the injectable vaccine group. Subjects receiving injectable vaccines have on average a much higher pre-challenge serum HI titre than those receiving the spray vaccine or placebo (Table 5). Compared to placebo and spray vaccinees, nearly three times more subjects vaccinated by injection had pre-challenge serum HI titres above eighty. If serological response is not a sensitive diagnostic criterion of infection in subjects with high pre-challenge serum HI titres (Beare *et al.*, 1969) assessing 'takes' by serology would bias results in favour of injectable vaccines.

The probable poor reliability of serology to diagnose infection at high pre-challenge serum HI titres is illustrated by the fact that both 'takes' at titres above eighty were diagnosed by virus isolation alone whereas this was the case in only two instances out of twenty-three 'takes' in subjects with titres of 13 or less.

From Table 5 it can be seen that there is an inverse relationship between pre-challenge serum HI titre and 'take' rate.

Second study

In contrast to the findings in the first study, in the second study 'take' rate was almost entirely dictated

TABLE 4. Findings in challenge study I. Infection rates in different groups

Vaccine group	Percentage of subjects with			χ^2-test against placebo (for 'take')
	⩾ One virus isolation	Serological response	'Take'	
Placebo ($n = 40$)	15·0	40·0	47·5	–
'Influvac Spray' lot A ($n = 41$)	0	19·5	19·5	$P = 0{\cdot}0075$
'Influvac Plain' lot I ($n = 40$)	5·0	5·0	7·5	$P < 0{\cdot}005$

TABLE 5. Findings in challenge study I. 'Take' rate and pre-challenge serum HI titre against challenge strain

Vaccine group	Percentage 'take' when pre-challenge HI			
	⩽ 13	14–40	41–80	> 80
Placebo (n = 40)	71·4† (n = 21)	100·0 (n = 1)	33·3 (n = 6)	8·3* (n = 12)
'Influvac Spray' lot A (n = 41)	50·0 (n = 14)	0 (n = 4)	9·1 (n = 11)	0 (n = 12)
'Influvac Plain' lot I (n = 40)	100·0 (n = 1)	50·0 (n = 2)	0 (n = 3)	3·0* (n = 34)

* One subject with virus isolation only; † two subjects with virus isolation only.

TABLE 6. Findings in challenge study II. Infection rates in different groups

Vaccine group	Percentage of subjects with			χ^2-test against placebo (for 'take')
	⩾ One virus isolation	Serological response	'Take'	
Placebo (n = 82)	73·2	6·1	75·6	–
'Influvac Spray' lot B (n = 48)	43·8	4·2	43·8	$P < 0·005$
'Influvac Plain' lot II (n = 46)	56·5	0	56·5	$P = 0·025$
'Cantacuzino Institute' lot 74–6 (n = 45)	60·0	0	60·0	$P = 0·06$

TABLE 7. Findings in challenge study II. 'Take' rate and pre-challenge serum HI titre against challenge strain

Vaccine group	Percentage 'take' when pre-challenge HI				
	⩽ 13	14–20	21–40	41–80	> 80
Placebo (n = 102)	76·7* (n = 60)	57·1 (n = 21)	70·0 (n = 10)	50·0 (n = 6)	100·0 (n = 5)
'Influvac Spray' lot B (n = 49)	40·6 (n = 32)	42·9 (n = 7)	66·7 (n = 3)	40·0 (n = 5)	50·0 (n = 2)
'Influvac Plain' lot II (n = 49)	61·1 (n = 18)	100·0 (n = 2)	58·8 (n = 17)	20·0 (n = 5)	71·4 (n = 7)
'Cantacuzino Institute' lot 74–6 (n = 51)	57·9 (n = 19)	40·0 (n = 5)	44·4 (n = 9)	72·7 (n = 11)	28·6 (n = 7)

* Two subjects with seroconversion only.

TABLE 8. Findings in challenge study II. Percentage positive specimens on days 1, 2 and 3 post-challenge in different groups

Vaccine group	Percentage positive specimens on Day 1	χ^2-test against placebo	Day 2	χ^2-test against placebo	Day 3	χ^2-test against placebo
Placebo	70·8 ($n = 89$)	–	30·0 ($n = 100$)	–	15·7 ($n = 102$)	–
'Influvac Spray' lot B	30·6 ($n = 49$)	$P < 0·005$	14·3 ($n = 49$)	$P = 0·03$	14·6 ($n = 48$)	$P = 0·48$
'Influvac Plain' lot II	37·5 ($n = 48$)	$P < 0·005$	31·3 ($n = 48$)	$P = 0·49$	10·4 ($n = 48$)	$P = 0·27$
'Cantacuzino Institute' lot 74–6	43·8 ($n = 48$)	$P < 0·005$	14·3 ($n = 49$)	$P = 0·03$	17·6 ($n = 51$)	$P = 0·47$

TABLE 9. Findings in challenge study III. Infection rates in different groups

Vaccine group	Percentage 'take' *	χ^2-test against placebo	Percentage 'take' when pre-challenge HI ⩽ 13	14–40	41–80	> 80
Placebo	22·7 ($n = 22$)	–	66·7 ($n = 3$)	20·0 ($n = 10$)	25·0 ($n = 4$)	0 ($n = 5$)
'Influvac Spray' lot A	16·7 ($n = 18$)	$P = 0·47$	50·0 ($n = 2$)	33·3 ($n = 6$)	0 ($n = 4$)	0 ($n = 6$)
'Influvac Plain' lot III	4·5 ($n = 22$)	$P = 0·09$	–	0 ($n = 1$)	0 ($n = 3$)	5·6 ($n = 18$)

* No virus isolation in this study.

by virus isolation (Table 6). In spite of the fact that the challenge type-B virus was antigenically very different from the strain in the inactivated vaccines, reasonable protection was obtained. Serological response to challenge was very poor and occurred only in the placebo and spray vaccine groups. Since the spray vaccine stimulates mostly local antibodies which presumably would make virus isolation more difficult, a challenge strain whose 'take' manifests itself mostly by virus isolation would bias results in favour of this vaccine. Although the differences are not significant the spray vaccine appeared better than the injectable ones in this study, either because of the possible bias in its favour or the fact that local immunity may be less specific than systemic immunity.

An unexpected finding in this study was the complete lack of correlation between pre-challenge serum HI titre and 'take' rate (Table 7). Two subjects with undetectable prechallenge serum antibodies seroconverted but no virus could be isolated from them. In Table 7 findings on subjects which could be definitely categorized in spite of some missing laboratory results are included, whereas only subjects with complete data are analysed in Table 6. This accounts for the differences in the sizes of the groups as reported in the two tables.

The incidence of virus isolations in this study was remarkably high. This might be because of the different isolation technique used in Rumania, or because of the nature of the Russian type-B vaccine.

Based on the percentages of positive throat swabs all vaccines were shown to give significant protection. Table 8 shows an analysis of findings according to day of collection. It can be seen that significant differences were mostly observed on the first day post-challenge when the isolation rate was the highest. On the second day only two vaccines gave significant protection whereas by the third day no differences were noted.

Third study

In this study in which virus isolation was attempted only on the second and third post-challenge days not a single virus was isolated. Thus, Table 9 shows the 'take' rates obtained by serological diagnosis alone. These were very low. Therefore, in view of the small number of subjects who completed the study, the protection observed with both vaccines was not statistically significant.

Again, in this study, there was some correlation between pre-challenge serum HI titre and 'take' rate (Table 9). Surprisingly, the only subject in the injectable vaccine group who was infected had a pre-challenge titre of over 80.

Fourth study

The results of this challenge study, in which virus was isolated from only two subjects, are shown in

TABLE 10. Findings in challenge study IV. Infection rates in different groups

Vaccine group	Percentage 'take' *	χ^2-test against placebo	Percentage 'take' when pre-challenge HI			
			⩽ 13	14–40	41–80	> 80
Placebo	32·0 (n = 50)	–	100·0 (n = 2)	46·2 (n = 26)	25·0 (n = 8)	0 (n = 14)
'Influvac Spray' lot A	20·6 (n = 34)	P = 0·19	–	37·5 (n = 8)	30·0† (n = 10)	6·3 (n = 16)
'Influvac Plain' lot IV	4·0 (n = 25)	P = 0·01	–	25·0 (n = 4)	0 (n = 6)	0 (n = 15)

* Only two virus isolations in this study: † one subject with virus isolation only.

TABLE 11. Findings in field trial. Clinical and serologically confirmed influenza rates in different groups

Vaccine group	Influenza incidence during outbreak			χ^2-test * against placebo
	Clinical diagnosis	Serological diagnosis A virus	Serological diagnosis B virus	
Placebo (n = 343)	2·3%	1·7%	0	–
'Influvac Spray' (n = 635)	2·0%	0·63%	0	P = 0·09
'Influvac Plain' (n = 348)	3·2%	1·1%	0	P = 0·37

* For serologically-confirmed type-A influenza.

TABLE 12. Observed protection rates in four challenge studies and one field trial

Vaccine group	Protection rates (underlined when P < 0·05) assessed in				
	Challenge study				Field trial
	I (n = 122)	II (n = 274)	III (n = 81)	IV (n = 124)	(n = 1326)
'Influvac Spray'	59%	42%	26%	36%	63%
'Influvac Plain'	84%	25%	80%	88%	35%
'Cantacuzino Institute'	–	21%*	–	–	–
Virus challenge	Heterologous type-A	Heterologous type-B	Homologous type-A	Homologous type-A	Wild type-A (probably homologous)

* P = 0·06

Table 10. There is a good inverse correlation between 'take' rate and pre-challenge serum HI titre. The protection conferred by the spray vaccine seems to be less than that by the injectable vaccine but the difference is not statistically significant.

Field trial

Table 11 records the outcome of the field trial. In spite of the fact that clinical influenza occurred in about 10% of the Zagreb general population during last winter's epidemic (Vodopija, 1975), the attack rates of both clinical and serologically confirmed influenza were very low in the study population. Therefore, although 1326 people participated in this trial, the observed vaccine-induced protections were not statistically significant.

Discussion

The results obtained in the four challenge studies and the field trial are summarized in Table 12. From these results it is clear that the protective efficacy of different vaccines against infection can be conclusively established in relatively small numbers of subjects by using highly attenuated live vaccines as challenge

viruses. These investigations have also demonstrated that intra-nasal and injectable inactivated influenza vaccines give protection against antigenically heterologous strains. Unfortunately, owing to the small number of subjects who got serologically-confirmed influenza in the simultaneously conducted field trial, it is not possible to decide whether this artificial method of assessment gives results comparable to those obtained in conventional field trials. Conclusions about the value of the method, therefore, can only be tentative.

In the field trial, the spray vaccines appeared to give better protection against type-A influenza than the injectable vaccine whereas the reverse was the case in the challenge studies with the type-A vaccines. On the other hand, when the type-B vaccine was the challenge strain the spray appeared better (Table 12). These paradoxical findings could possibly be explained by the different primary criteria used to diagnose infection in the various studies, namely, illness, virus isolation and serological response. Thus, when challenge 'takes' are mostly assessed by virus isolation, as was the case with the type-B vaccine, an injected vaccine may be at a disadvantage compared to a sprayed vaccine but when 'takes' are assessed mostly by serological response, as with the type-A vaccines, the reverse may be the case.

Contrary to the findings with the type-A vaccines (Tables 5, 9 and 10) the striking lack of correlation between pre-challenge serum HI titre and the 'take' rate of the type-B vaccine (Table 7) also requires an explanation. The type-B vaccine was probably very attenuated, causing both minimal inflammation and serum antibody responses, compared to the type-A vaccines. This conclusion was supported by the clinical observation of a lower incidence and severity of throat injection among the type-B vaccinees. Thus, in these subjects, transudation of circulating antibodies would occur to a limited extent, possibly accounting for this lack of correlation. With the less attenuated type-A vaccines, transudation through inflamed mucosae may occur and this could be the reason for the observed correlation between serum antibodies and 'take' rate.

Thus, since local and systemic antibody levels can vary independently (Tyrrell *et al.*, 1973), for meaningful comparison of vaccines administered by spray and injection respectively, the challenge strain should ideally cause disease, as in field studies, or cause both virus shedding and serological response, if it is highly attenuated, as in the present challenge studies. However, when vaccines under test are administered by the same route any live vaccine (challenge) strain with a reasonably high 'take' rate should yield valid results. This convenient method of comparing the efficacy of different vaccines deserves further attention.

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