Influenza subunit vaccine: antibody responses to one and two doses of vaccine and length of response, with particular reference to the elderly

JOHN S MacKENZIE

British Medical Journal, 1977, 1, 200-202

Summary

Antibody responses to subunit influenza vaccine prepared against A2/England/42/72 (H3N2) were studied in 69 volunteers aged 60 and over and 231 aged 59 and below over 12 months in 1973 and 1974. After two doses of vaccine seroconversion frequencies and geometric mean haemagglutination-inhibition (HI) titres were higher in the elderly, but no differences were observed between the two groups in the length of their responses. Sixteen (23%) of the elderly volunteers seroconverted only after receiving a second dose of vaccine or seroconverted twice after receiving both doses of vaccine. It was considered justifiable, therefore, to recommend the continuation of a two-dose schedule for patients in a high-risk category. Within 30 weeks of vaccination 87 (29%) volunteers had considerably reduced HI titres (≤ 48), which might indicate potential susceptibility to influenza during an epidemic, and the number had risen to 132 (44%) by 50 weeks. It was suggested that high-risk patients should receive annual vaccination two to four months before the possible epidemic period.

Introduction

Generally influenza immunisation is recommended to be available each year to people in high-risk categories, defined as those with chronic bronchopulmonary or cardiovascular-renal disease and those aged over 65.¹ Although at one time a two-dose vaccination schedule was advocated, recent studies have suggested that when minor antigenic changes occur in the epidemic virus strain a second dose of homologous virus vaccine may not produce a significant increase in the geometric mean haemagglutination-inhibiting (HI) antibody titre beyond that induced by the preceding initial dose, and may therefore be of limited additional value.²⁻⁵ A two-dose schedule, however, would still be necessary for children without prior antigenic stimulation and for adults if a major antigenic shift occurred in the epidemic virus. None of the earlier reports has considered the potential disadvantages that might exist with a single dose schedule in "high-risk" category patients or the effect on the length of the response.

This report analyses the immune responses elicited by one or two doses of subunit influenza vaccine in a group of elderly volunteers (aged 60 and over) and compares their responses with those in a group of younger people (aged 15 to 59). The preliminary results of part of this study have been published elsewhere.⁶

Methods

The trial was undertaken between July 1973 and July 1974. Three hundred volunteers were drawn from three places in Western Australia; Busselton, Collie, and Perth. All volunteers had prevaccination HI titres of 192 or less. The vaccine used was influenza virus (subunit) vaccine (Commonwealth Serum Laboratories, Melbourne), which contained 16 000 haemagglutinating (HA) units of A/England/42/72 (H3N2) and 8000 units of B/Roma/1/67. Two doses were administered by deep subcutaneous injection four weeks apart. The volunteers had a blood sample withdrawn immediately before receiving each dose of vaccine and seven, 30, and 50 weeks after vaccination. The volunteers were grouped by age into two groups: 69 patients aged 60 or over (60-79; mean 66.5 years) and 231 aged 59 or below (15-59; mean 42.5 years).

Serum samples were separated and stored at -20°C until assayed. HI titrations were performed by standard procedures after the removal of non-specific inhibitors by treatment with receptor-destroying enzyme.⁷ A fourfold or greater rise in HI antibody titre was assumed to indicate seroconversion.

Results

The seroconversion frequencies after vaccination are shown in table I. A significantly higher proportion of the volunteers aged 60 and over seroconverted after receiving both doses of vaccine than those aged 59 and below ($\chi^2_1 = 5.37$; $P < 0.025$). Although most subjects in the two age groups responded after receiving their first dose of vaccine, 9 (13%) of the elderly group and 27 (12%) of those aged 59 and below failed to respond until the second dose. Moreover, some people who had seroconverted after the first dose of vaccine also showed a fourfold or greater rise in HI titre after the second dose (10% of those aged 60 and above; 7% of those aged 59 and below).

TABLE I—Seroconversion frequencies after administration of subunit influenza vaccine

Vaccine group (by age)	No of vaccinees	No who seroconverted after:			Seroconversion %
		1st dose only	2nd dose only	Both doses	
60-79	69	47	9	7	91
15-59	231	140	27	16	79

This secondary rise was more than fourfold in three cases. No significant correlation could be found between prevaccination HI titres and the proportion of volunteers who seroconverted only after receiving the second dose of vaccine (see figure).

Postvaccination geometric mean HI antibody titres for the two groups of subjects after each dose of vaccine (four and seven weeks) and subsequently after 30 and 50 weeks are shown in table II. Higher geometric mean titres were observed in the elderly group, but there was little difference between the two age groups in the length of their responses. To examine the responses in greater detail the highest post-vaccination titre of each subject (at four or seven weeks after vaccination) was analysed in terms of prevaccination titre (tables III and IV). As a measure of longevity, the proportion of people with titres of ≤ 48 at 30 and 50 weeks are included in each table. About 60% of the volunteers in both age groups showed substantial antibody titres, 384 or higher, but a significant proportion of subjects with little or no residual antibody before vaccination (titres of ≤ 24) responded with titres of

192 or less. Furthermore, regardless of age, 32% of the subjects with little or no residual antibody before vaccination were found to have HI titres of 48 or less 30 weeks after vaccination, and 51% had similarly low titres at 50 weeks. Most subjects with prevaccination titres of ≥ 48 , however, still retained significant levels of HI antibody (titres of ≥ 96) 50 weeks after vaccination.

Discussion

Over two-thirds of the excess deaths in the United States and Europe from the influenza epidemics of 1957-8 and 1959-60 occurred in people aged 65 and over.^{8,9} This age group, therefore, is one of the high-risk categories for whom influenza immunisation is strongly recommended. In this study volunteers aged 60 and over were included in the elderly group, and their antibody responses compared with those of a group comprising all other ages (15-59). A significantly higher proportion of those in the elderly group seroconverted after receiving the two doses of vaccine, and they showed slightly raised geometric mean HI antibody titres compared with the 15-59 age group (tables I and II). In a previous report using the same data as this study,⁷ it

Percentage of people seroconverting after receiving influenza vaccination. Hatched and white columns represent vaccinees aged 60 and over. Black and stippled columns represent vaccinees aged 59 and below. Hatched and black sections show percentage of vaccinees seroconverting after first dose. Numbers above each column indicate number of subjects vaccinated.

TABLE II—Geometric mean HI antibody titres after administration of subunit vaccine

Vaccine group (by age)	Geometric mean HI titres:				
	Before vaccination	After vaccination			
		4 weeks	7 weeks	30 weeks	50 weeks
60-79	12	371	475	228	161
15-59	9	217	326	189	120

TABLE III—Postvaccination HI titres of vaccinees aged 60-79 who showed seroconversion and percentage with titres of ≤ 48 30 and 50 weeks after vaccination

Prevaccination titre	Postvaccination titres (highest titre at 4 or 7 weeks)						% Vaccinees with titres of ≤ 48 at*:	
	24	48	96	192	384	≥ 768	30 Weeks	50 Weeks
	≤6	2	3	3	4	7	43	57
12	3	1	3	3	4	7	35	60
24			3	2	2	7	20	39
48				0	2	2	0	0
96					0	4	0	0
192						2	0	0
Total							29	45

*Samples were not obtained from all vaccinees.

TABLE IV—Postvaccination HI titres of vaccinees aged 15-59 who showed seroconversion and percentage with titres of ≤ 48 30 and 50 weeks after vaccination

Prevaccination titre	Postvaccination titres (highest titre at 4 or 7 weeks)						% Vaccinees with titres of ≤ 48 at*:	
	24	48	96	192	384	≥ 768	30 Weeks	50 Weeks
	≤6	21	15	7	12	22	46	65
12	2	9	8	9	9	26	12.5	37
24		3	1	7	7	13	6	7
48			2	1	1	11	12.5	0
96				0	0	4	0	0
192						4	0	0
Total							29	43

* Samples were not obtained from all vaccinees.

was observed that the seroconversion frequencies and geometric mean titres were depressed in subjects aged 20 and below. Possibly therefore the younger cohort in the 15-59 age group caused a reduction in the geometric mean titres through a dilution effect. Such an explanation is at variance, however, with a report by Marine and Thomas,¹⁰ who found that younger subjects responded better than older ones to a vaccine prepared from A/Aichi/2/68(H3N2), although their age grouping was considerably different to the grouping in this study, and their vaccine was a zonally purified whole-virus vaccine.

Most subjects in both age groups seroconverted after receiving their first dose of vaccine, and a second dose did not significantly increase their geometric mean titres (the titre increased 1.3-fold in subjects aged 60 and over and 1.5-fold in those aged 59 and below). These results, therefore, tended to agree with those in other reports using aqueous whole virus^{2,3} and subunit vaccines,⁵ which had suggested that a two-dose schedule was unnecessary in normal healthy adults. Nevertheless, 13% of the elderly group seroconverted only after receiving the second dose, and an additional 10%, who had previously responded after the first dose, showed further seroconversion after their second dose, regardless of prevaccination titres. All possible measures should be taken to protect people at high-risk during influenza epidemics, and if a second dose of vaccine will provide protection or increased antibody levels for a further 23% of people in high-risk categories then a two-dose vaccine schedule is ethically justifiable and should be recommended automatically. Although a two-dose schedule also resulted in additional protection for 18.6% of healthy adults below the age of 60, a single dose should suffice provided they do not have conditions that would place them in other World Health Organisation high-risk categories, such as bronchopulmonary or cardiovascular disease or other serious chronic debilitating diseases.

Epidemiological studies¹¹ and challenge studies¹² have suggested that HI titres of 24-40 may provide an indication of protection against infection. Serological studies, however, have suggested that pre-epidemic titres of 48 may not fully protect, although the data could not distinguish between clinical and subclinical infections.⁷ Titres of 48 or less were therefore chosen as a measure of longevity of antibody after vaccination and potential susceptibility to infection. A small proportion of subjects in both age groups responded poorly to vaccination with titres of 96 or less, but most showed antibody titres of 192 or greater. Regardless of their age group, however, 32% of subjects who had little or no detectable immunity before vaccination (titres of ≤ 24) had titres of 48 or less 30 weeks after vaccination, and 51% had titres of 48 or less 50 weeks after vaccination (tables III and IV). All but two people with prevaccination titres of 48 or higher still retained substantial levels of antibody (titres of ≥ 96) 50 weeks after vaccination. If the volunteers in this study are taken as representing the general population, and without information on their antibody state, then about 29% of vaccinated people can be expected to have titres of 48 or less within 30 weeks of vaccination, and 44% within 50 weeks of vaccination.

If an HI titre of 48 or less indeed indicates potential susceptibility then the results of this study suggest that people in a high-risk category should be strongly encouraged to undertake a two-dose vaccination schedule annually, especially when a new influenza strain has arisen by antigenic drift, and they should receive their first dose of vaccine within two to four months of the normal seasonal epidemic period. It may be fallacious to judge the effectiveness of a vaccine in terms of antibody persistence within a population by the height of geometric mean HI titres. Such titres may reflect an inflated level of immunity by assessing a population rather than the antibody status of individuals within the population.

I thank Mrs Isobel MacKenzie and Mrs Julie Lloyd for their excellent technical help. This study was supported by grants from the National Health and Medical Research Council of Australia and Smith Kline and French Laboratories (Australia) Ltd.

References

- ¹ Eickhoff, T C, *Journal of Infectious Diseases*, 1971, **123**, 446.
- ² Howells, C H L, Evans, A D, and Vesselina-Jenkins, C, *Lancet*, 1973, **1**, 1436.
- ³ Picciotto, L, *et al*, *British Medical Journal*, 1973, **4**, 169.
- ⁴ Hobson, D, Baker, F A, and Curry, R L, *Lancet*, 1973, **2**, 155.
- ⁵ Feery, B J, *et al*, *Medical Journal of Australia*, 1976, **1**, 186.
- ⁶ MacKenzie, J S, *Medical Journal of Australia*, 1976, **1**, 546.
- ⁷ MacKenzie, J S, *et al*, *Journal of Hygiene*, 1975, **75**, 425.
- ⁸ Dauer, C C, and Serfling, R E, *American Review of Respiratory Diseases*, 1961, **83**, suppl p 15.
- ⁹ Eickhoff, T C, Sherman, I L, and Serfling, R E, *Journal of the American Medical Association*, 1961, **176**, 776.
- ¹⁰ Marine, W M, and Thomas, J E, *Postgraduate Medical Journal*, 1973, **49**, 164.
- ¹¹ Evans, A S, Jeffrey, C, and Niederman, J C, *American Journal of Epidemiology*, 1971, **93**, 463.
- ¹² Hobson, D, *et al*, *Journal of Hygiene*, 1972, **70**, 767.

(Accepted 18 November 1976)

Intrauterine deposition of calcium on copper-bearing intrauterine contraceptive devices

CHRISTINE GOSDEN, ALAN ROSS, NANCY B LOUDON

British Medical Journal, 1977, **1**, 202-206

Summary

Copper-bearing intrauterine contraceptive devices (IUDs) removed after various times in utero were examined by scanning electron microscopy and x-ray microanalysis of the elements present. As time in utero increased these devices became increasingly calcified. This calcification may limit the release of copper from the devices and decrease the specific contraceptive effectiveness of copper over an inert plastic device. Conversely, any teratogenic effects attributable to the copper may decrease with time in utero and depend on the extent of calcification.

Even though the amount of copper in the device is not significantly diminished after two years, devices should not remain in situ for over two years because calcium accumulation probably prevents further diffusion of copper. Calcification can begin as early as six months after insertion. Consequently a careful review of the amount of time a copper-containing IUD should be left in situ should be undertaken.

Introduction

Winding copper wire round an inert plastic intrauterine device (IUD) was found to improve its contraceptive efficiency and

reduce side effects.¹ Available data seemed to indicate an inverse relation between the surface area of the copper and the pregnancy rate.² It is not known how plastic IUDs work, although it has been suggested that they somehow prevent implantation of a fertilised ovum by direct local action on the endometrium. The decrease in pregnancy rate when copper is added suggests an additional contraceptive effect, possibly related to toxic effects on sperm, ovum, and embryo; the effect of copper on endometrial biochemical pathways, thus disturbing implantation; or local inflammatory responses with macrophage stimulation, leucocyte infiltration, and changes in cervical mucus.^{3,4} Copper released from these devices significantly increases copper concentrations in the intrauterine fluid in the first 12 months, but it does not increase serum copper or caeroplasm concentrations.^{5,6}

Hagenfeldt⁵ found a linear relation between the release rate of copper and the duration of use of the device over the first year. On the graph, however, the line did not pass through the origin, which suggests that there is a higher release rate during early cycles of IUD use. Copper concentrations in the proliferative endometrium were increased during the first three cycles but not thereafter, and the copper concentration of the cervical mucus also increased considerably during the first six treatment cycles, especially during the proliferative phase. The enhanced contraceptive efficiency of the copper IUDs compared with inert plastic IUDs is greatest in this initial period.

Hagenfeldt also measured the amount of copper on the devices by dissolving the metal in concentrated HNO₃ and estimating the copper colorimetrically. Intrauterine release rates varied from 7.4 to 13.3 mg of copper in the first year. Given a constant release rate, an average of 28.7 µg of copper was therefore released each day. Tatum⁷ suggested that a device's effective life is determined by the time taken to reduce the copper surface area below that required to provide acceptable contraceptive action. Using Hagenfeldt's figures for copper loss, he estimated that this point would be reached when 40 mg of copper had been released. A conservative estimate of the life of a device might therefore be about two years, but this could be extended and a third or fourth year of effective use obtained.

The suggestion that contraceptive efficiency is reduced when

MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh

CHRISTINE GOSDEN, BSC, PHD, member of scientific staff
ALAN ROSS, FIMLS, MIBIOL, chief research officer

Family Planning Services, Lothian Health Board

NANCY B LOUDON, MB, CHB, senior medical officer