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Comparative study of immunogenicity of split, intradermal and MF59-adjuvanted influenza vaccines in elderly institutionalized subjects

Michela Basileo[1], Anna Maria Iorio[1]*, Guido Bartolini[2], Cinzia Bianchini[3], Giuseppe Menculini[4], Paolo Tozzi[5], Barbara Camilloni[1]

[1]Dept. Exp. Med. University of Perugia, Italy; [2]Opera Pia B. Castori, Foligno, Italy;[3]Coop. Soc. A.I.D.A.S. Terni, Italy; [4]RP Bittoni C. Pieve, Perugia, Italy; [5]Azienda USL2, Foligno, Italy

Abstract

The reduced immunogenicity and effectiveness of influenza vaccines in subjects presenting high risk of influenza-related complications, hospitalization and death, led the innovative drive to search for new strategies to implement the immune response elicited by influenza vaccines including addition of adjuvants, and use of alternative routes of antigen delivery.In this study we evaluated and compared the immune antibody response induced in 252 elderly volunteers living in nursing homes after immunization with three different 2012-2013 seasonal trivalent inactivated influenza vaccines: a conventional split vaccine (n=26), and two potentiated vaccines (a subunit vaccine adjuvanted with MF59 (n=137) or a split vaccine administered intradermally (n=89)), specially licensed for elderly people. Haemagglutination inhibiting (HI) antibody titers were assessed in blood samples collected before and one month after vaccination.The results were evaluated as increase in HI titers found comparing pre- and post-vaccination sera and according to the Committee for Medicinal Products for Human Use (CHMP) criteria for approval of influenza vaccines in the elderly. Significant antibody increases and fulfillment of all the three CHMP requirements were observed against A/H3N2 and B antigens following immunization with the two potentiated vaccines. After immunization with conventional vaccine responses were lower against A/H3N2 and equivalent against the B antigen. The two potentiated vaccines induced significant antibody increases against A/H1N1 antigen, however, only one of the CHMP criteria was reached. The HI antibody increases after conventional vaccine were significant only for the geometric mean titer and none of the CHMP criteria was fulfilled. The antibody responses induced by the two potentiated vaccines against the three vaccine

* Corresponding author. Tel.: +39-075-5858249; fax: +39-075-5858415.
E-mail address: annaiorio42@gmail.com

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antigens wereequivalent although post-vaccination titers against the B antigen tended to be higher in subjects vaccinated with intradermal vaccine than in individuals receiving MF59-adjuvanted vaccine.
In conclusion the use of MF59 adjuvant and intradermal vaccination appear to be appropriate strategies to address the challenge of declining immune response in the elderly after influenza vaccination.

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*Keywords:*immunogenicity; elderly institutionalized people; split influenza vaccine; MF59-adjuvanted influenza vaccine; Intradermal influenza vaccine.

1. Introduction

Annual influenza infection is a major cause of substantial increases of morbidity and mortality particularly in the elderly and in the other risk groups. Influenza vaccination is considered the major tool of preventing clinical disease and associated complications. However because of the effects of "immunosenescence", i.e. the age correlated decline of immune function[1,2], influenza vaccines for elderly must be adapted to optimize the immune response to vaccination.

Several approaches have been investigated and in the last years two potentiated influenza vaccines, Fluad® and Intanza® 15mcg were developed and licensed for use in the elderly. Fluad® is an MF59-adjuvanted subunit vaccine administered intramuscularly, Intanza®15mcg is a split non-adjuvanted vaccine administered intradermally, i.e. in the immune-rich environment of the dermis. The two vaccines have been found to have an acceptable safety profile and to induce in most instances a higher immune response as compared with conventional vaccines[3-6].

In Italy Fluad® was commercialized in 1997 and Intanza®15mcg is available from the 2010-11 Winter season. The two potentiated vaccines and conventional inactivated vaccines are freely offered by the Italian Health Ministry for vaccination of the elderly high-risk group[7].

Since it is important to recognize any relevant advantage among the different commercialized influenza vaccines, the aim of our study was to compare in the 2012-13 Winter season the ability of inducing antibody responses of Vaxigrip® a conventional inactivated split vaccine, with that of the two potentiated vaccines, Fluad® and Intanza®15mcg, in elderly institutionalized volunteers.

2. Materials and methods

2.1. Study design and vaccination

During the 2012-13 Winter season 252 elderly people living in 7 different nursing homes located in Umbria (Italy), received one dose of one of the influenza vaccines freely offered by the Public Health Authorities of Umbria to the high risk group of elderly people. Twenty six were vaccinated with an intramuscular conventional split vaccine (Vaxigrip®, Sanofi-Pasteur MSD, France) (IM), and 226 with a potentiated vaccine (137 with an intramuscular subunit vaccine adjuvanted with MF-59 (Fluad®, Novartis, Vaccines, Italy) (IM-MF59) and 89 with an intradermal split vaccine (Intanza 15 mcg®, Sanofi-Pasteur MSD, France) (ID)).
Each dose of the three vaccines contained 15 mcg of A/Victoria/361/11 (H3N2), A/California/7/09 (H1N1), and B/Wisconsin/1/10 respectively in 0.5ml (IM and IM-MF59) and 0.1 ml (ID).
Serum samples were collected before and 1 month after vaccination and stored at -20°C to simultaneously assess the immunogenicity.
Information about the health status of the elderly people was obtained for each volunteer using data reported in the Italian VAOR (Valutazione Anziano Ospite di Residenza) schedule.
The study was conducted according to the declaration of Helsinki and Good Clinical Practices. Since all the three vaccines were assigned to the nursing homes of Umbria for the vaccination of elderly residents within the annual influenza vaccination campaign and sera were leftover sera from samples collected for clinical routine control, the study did not need to be registered as a formal trial.

2.2. Vaccine immunogenicity

Antibody responses were measured by a standard microtiter haemagglutination inhibition test (HI) using heat-inactivated (56°C for 30') sera treated with receptor-destroying enzyme to remove non-specific inhibitors and 0.5% turkey erythrocytes[8].

The antigens tested were the three vaccine strains cultured in Madin-Darby canine kidney cells.

Vaccine immunogenicity was assessed by comparing antibody titers in blood samples, collected before and 1 month after vaccination, as seroprotection rate (the proportion of subjects showing an HI titer ≥40, considered to be associated with protection from influenza infection)[9] and as geometric mean titers (GMT) (the first dilution for antibody titration was 1:10 and any titer <10 was considered 5 for GMT calculation).

The results were evaluated according to the requirements of the Committee for Medicinal Products for Human Use (CHMP) criteria for approval of influenza vaccines in the elderly (i.e. post-vaccination seroprotection rate ≥60%, mean fold increase (MFI) of GMT (ratio of post-immunization titer to pre-immunization titer) ≥2 and seroconversion rate (subjects with a fourfold or greater increase in titer in pre-vaccination serum positive people or from <10 to ≥40 in seronegative volunteers) ≥30%)[10].

2.3. Statistical analysis

Differences in the frequency of qualitative variables were analyzed by Student's t-test (comparison of geometric mean titers) and Chi-square test (comparison of number of people with protective antibody titers and seroconversion).

3. Results

3.1. Characteristics of the study subjects

Table 1 shows the demographic characteristics and the health status of the 252 institutionalized volunteers aged ≥60 years and subdivided into three groups according to the type of influenza vaccine received.

The three groups were slightly different for some parameters since the mean age and the number of people with underlying diseases were lower in the group of elderly receiving the conventional vaccine as compared with people vaccinated with the potentiated formulations. Moreover the percentage of people with cardiovascular diseases was higher in volunteers vaccinated with Intanza® 15mcg.

Table 1. Baseline characteristics of the elderly institutionalized subjects participating in the study immunized with different influenza vaccines recommended for the 2012-2013 Winter season.

	Vaxigrip® IM (n= 26)	Fluad® IM-MF59 (n= 137)	Intanza® 15mcg ID (n= 89)
Mean age	74.9 [A,b]	85.1	84.6
(Range)	(60-90)	(60-102)	(60-103)
% Underlying diseases*	69.3 [A,b]	89.1	89.8
% Cardiovascular diseases	19.2	20.4	43.2 [b,C]
% Diabetes mellitus	19.2	13.9	11.4
% Respiratory diseases	19.2	10.2	26.1 [C]
% Cancer	7.7	3.6	6.8
% Dementia	46.1	45.2	36.4
% Chronic use of drugs**	100	98.5	98.9

*: more than one risk status was possible for each subject
**: drugs most frequently used were antihypertensive/inotropic drugs and benzodiazepines
A: p<0.01; a: p<0.05 comparing IM with IM-MF59
b: p<0.05 comparing IM with ID
c: p<0.05 comparing IM-MF59 with ID

3.2. HI antibody response induced following immunization with the three influenza vaccines.

Table 2 reports the results obtained from comparing HI titers in sera collected before and one month after vaccination.

Pre-vaccination HI titers against the three different vaccine strains were similar and a statistically significant increase of pre-vaccination titers, evaluated as percentage of protected people and as GMT values, against the three different vaccine strains, was observed in the three groups of volunteers vaccinated with different influenza vaccines, except for the seroprotection rate against A/H3N2 strain in IM group.

One month after vaccination no significant differences were observed across vaccine groups against A/H3N2 and A/H1N1 vaccine antigens, while the post-vaccination GMT value against the B antigen was higher in the ID group as compared with the IM-MF59 group (49.0 vs 41.9, $p<0.05$).

Although the pre-requisite of at least 50 persons per group was not met in the group vaccinated with the conventional vaccine (n=26) and although there are some disagreements on the identification of a single threshold (HI titer ≥40) for defining protection[11], the serological results observed one month after vaccination were also evaluated according to the CHMP criteria for approval of influenza vaccines in the elderly[10].

Considering separately the three vaccine strains, the results observed against the A/H3N2 antigen showed that the two potentiated vaccines (IM-MF59 and ID) satisfied all three criteria whereas the values of MFI and the seroconversion rate of the IM group were lower than the requested (1.7 and 19.2 respectively).

The responses induced against the A/H1N1 antigen were less satisfactory since none of the three criteria was met with the conventional vaccine and only the MFI values with IM-MF59 and ID vaccine achieved the requested limit (2.1 and 2.0 respectively).

All three requirements were satisfied for the B antigen in the three vaccine groups: the MFI values ranged from 2.6 to 3.5, the seroprotection rate from 61.5 to 71.9% and the seroconversion rate from 32.8 to 42.7%.

Table 2. HI antibody response against the three vaccine antigens (A/Victoria/361/11 (H3N2), A/California/7/09 (H1N1), and B/Wisconsin/1/10) in elderly institutionalized volunteers following immunization with three 2012-13 different influenza vaccines (Vaxigrip® (IM), Fluad® (IM-MF59) and Intanza®15mcg (ID)).

Vaccine antigen	Vaccine (n)	Seroprotection [CI 95%]		GMT [CI 95%]		MFI [CI 95%]	Seroconv. [CI 95%]	CHMP criteria satisfied
		Pre-vacc.	1 month	Pre-vacc.	1 month			
A/H3N2 A/Victoria/361/11	IM (26)	38.5 [22.8-57.0]	65.4 [46.7-80.3]	23.2 [9.8-55.2]	40.6* [17.6-93.5]	1.7 [1.1-2.8]	19.2 [8.7-37.3]	1/3
	IM-MF59 (137)	42.3 [35.7-49.2]	70.8** [64.2-76.6]	26.4 [19.1-36.4]	62.8** [45.1-87.5]	2.4 [1.9-3.0]	30.0 [24.0-36.6]	3/3
	ID (89)	43.8 [34.9-53.1]	74.2** [65.3-81.4]	23.6 [15.7-35.3]	61.9** [40.5-94.5]	2.6 [1.9-3.6]	33.7 [25.6-42.9]	3/3
A/H1N1 A/California/7/09	IM (26)	7.7 [2.2-23.6]	19.2 [8.7-37.3]	6.5 [4.4-9.7]	10.5* [5.7-19.3]	1.6 [1.0-2.6]	11.5 [4.1-28.4]	0/3
	IM-MF59 (137)	15.3 [11.0-20.9]	40.1** [33.6-47.0]	10.1 [7.9-12.7]	20.6** [15.2-27.9]	2.1 [1.6-2.5]	22.6 [17.4-28.8]	1/3
	ID (89)	12.4 [7.5-19.8]	32.6** [24.6-41.8]	8.6 [6.6-11.1]	17.0** [11.7-24.6]	2.0 [1.5-2.6]	18.0 [11.9-26.2]	1/3
B/Wisconsin/1/10	IM (26)	15.4 [6.3-32.7]	61.5** [43.0-77.2]	13.0 [8.4-20.4]	41.1* [19.8-85.3]	3.1 [1.5-6.7]	38.5 [22.8-57.0]	3/3
	IM-MF59 (137)	26.3 [20.7-32.7]	64.2** [57.4-70.5]	16.2 [13.3-19.8]	41.9**[a] [32.7-53.6]	2.6 [2.1-3.2]	32.8 [26.7-39.5]	3/3
	ID (89)	22.5 [15.7-31.1]	71.9** [62.9-79.4]	14.0 [10.4-18.8]	49.0** [34.2-70.2]	3.5 [2.6-4.7]	42.7 [33.9-52.0]	3/3

*: $p<0.05$; **: $p<0.01$ comparing pre and post-vaccination data

a: $p<0.05$ comparing IM-MF59 with ID

4. Conclusions

The present study was carried out to estimate and compare the HI antibody response induced in institutionalized elderly subjects after vaccination with a conventional (Vaxigrip®) (IM) or with two different potentiated (Fluad® (IM-MF59) or Intanza®15mcg (ID)) influenza vaccines licensed for use in elderly people. The three vaccines were seasonal trivalent influenza vaccines commercially available for the 2012-2013 Winter season (A/Victoria/361/11 (H3N2), A/California/7/09 (H1N1), and B/Wisconsin/1/10). The study included 252 elderly subjects and 10.3% of them received the traditional IM vaccine, 54.4% IM-MF59 and 35.3% ID.

In agreement with other studies, both potentiated vaccines were found to be capable of inducing higher or similar immune responses in the elderly when compared to a conventional non-potentiated influenza vaccine[3-6]. In the present study one month after vaccination the HI antibody response was higher against A vaccine antigens after vaccination with the two potentiated vaccines as compared with the conventional one. Indeed, more frequently significant increases in seroprotection and GMT values against the A antigens were induced, although the differences were not statistically significant, in the IM-MF9 and ID groups as compared with the IM group (Table 2). The fulfillment of the three CHMP criteria confirmed these results, since three criteria against A/H3N2 and one against A/H1N1 were met in people vaccinated with the potentiated vaccines as compared with one criteria against A/H3N2 and none against A/H1N1 antigen in the IM group. Responses against the B antigen were similar, since increases in HI antibody titers and fulfillment of all three CHMP criteria was found in all the three groups examined.

Considering the results obtained after immunization with the two potentiated vaccines, a direct comparison of the HI antibody responses induced by the same two potentiated vaccines (IM-MF59 and ID) in elderly people was previously reported by Van Damme[12] for the 2007-2008 Winter season and by Scheifele et al.[6] for the 2011-2012 Winter season. The results of Van Damme et al.[12] and of Scheifele et al.[6] differ under some aspects from ours. Considering the HI responses against the two A vaccine strains, post-vaccination GMT values against the A/H3N2 strain were higher in the IM-MF59 as compared with the ID group both in Van Damme et al.[12] and Scheifele et al.[6], whereas our data did not evidence differences (Table 2). In accordance with Van Damme et al.[12] we observed similar responses against the A/H1N1 strain, whereas Scheifele et al.[6] found higher post-vaccination GMTs in volunteers vaccinated with IM-MF59 as compared with ID group. Both Van Damme and Scheifele found that antibody responses after vaccination with IM-MF59 and ID were similar when assessed by single radial hemolysis (SRH) assay, a method recognized with HI by CHMP for the evaluation of influenza vaccines.

Examining the post-vaccination GMT values against the B strain, the results obtained by Scheifele et al.[6] could not be evaluated because of the high baseline antibody values precluding meaningful response assessment. Van Damme et al.[12] found similar post-vaccination GMT in the two groups, whereas our data (Table 2), evidenced a slightly higher GMT value in the ID group as compared with IM-MF59 (49.0 vs 41.9 $p<0.05$) although the values of seroprotection and seroconversion were similar and all three CHMP requirements were met in the two vaccine groups.

Our study had several limitations. The most important are that our observations may apply only to frail seniors living in care facilities and that the three groups were not fully comparable. However, since institutionalized people represent a significant target group for influenza vaccination, is important to know their response to potentiated vaccines. The senior group vaccinated with conventional vaccine showing the lower response was younger and with less underlying diseases as compared with elderly vaccinated with potentiated vaccines, two characteristics known to positively influence immune response.

In conclusion our data evidenced that the use of MF59 adjuvant and of intradermal vaccination appear to be appropriate strategies to address the challenge of the declining immune response in the elderly. However, they underline the need of studies for new improved influenza vaccines, since, as previously found, the responses against the three vaccine antigens were different and for the 2012-2013 Winter season resulted not satisfactory against the A/H1N1 antigen.

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