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Safety and Immunogenicity of Conventional Subunit and MF59-adjuvanted Influenza Vaccines in Human Immunodeficiency Virus-1-seropositive Patients

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In this study of influenza vaccination, 37 human immunodeficiency virus (HIV)-1-seropositive patients were randomized to receive either a vaccine with a conventional subunit or one adjuvanted with MF59. Blood samples were collected at the time of vaccination, and then 30 and 180 days later, to evaluate immunogenicity, CD4+ T-lymphocyte count and HIV-1 RNA levels. Seroconversion rates against the three viral strains included in the vaccine ranged between 44% and 72% and 53% and 68% for the adjuvanted vaccine and the subunit vaccine, respectively. Other criteria of the European Medicines Evaluation Agency were also met. Vaccination was not associated with serious adverse events. Local and systemic effects were mild and of short duration. CD4+ T-lymphocyte counts and viraemia levels were not negatively affected by vaccination. These results confirmed the safety and immunogenicity of these currently available vaccines in HIV-1-seropositive patients, thus supporting the recommendation for influenza immunization in this high-risk category.

KEY WORDS: Influenza vaccine; Human immunodeficiency virus; Safety; Immunogenicity; CD4+ T-lymphocytes; Human immunodeficiency virus-1 RNA

Introduction

Influenza vaccination in high-risk subjects, such as those ≥ 65 years of age and those with chronic respiratory, cardiac or renal disease or immunodepression, results in a considerable reduction in morbidity and mortality rates, with obvious benefits not

only in terms of health but also from socio-economic perspectives.[1–3] Influenza infection in patients positive for human immunodeficiency virus (HIV) may be particularly severe: the influenza virus may continue to replicate for weeks or months, prolonging its shedding and increasing the risk of complications, hospitalization and death.[4–9]

Influenza vaccination of high-risk patients is recommended by the World Health Organization (WHO) and is current practice in several countries,[2,10,11] but its use in HIV-1-infected subjects is not yet accepted worldwide. This is because of the possible negative effect of vaccination on viraemia levels and the CD4+ T-lymphocyte count, together with the inadequate immune response induced by the vaccine in severely immunodepressed subjects.[12–14] The development of adjuvanted influenza vaccines, which have already been shown to have excellent immunogenicity and safety profiles in the elderly and in children, could improve the prevention of influenza in HIV-1-seropositive patients, even if the response to vaccination is still far from optimal when compared with that achieved in healthy adult subjects.[15,16]

The aim of this study was to evaluate and compare the tolerability and immunogenicity of two influenza vaccines currently on the market – one with a conventional subunit and the other adjuvanted with an oil–water emulsion – in a cohort of adult HIV-1-seropositive subjects. Safety was assessed by evaluating the effect of vaccination on plasma levels of viraemia (HIV-1 RNA) and the CD4+ T-lymphocyte count.

Patients and methods

STUDY DESIGN AND VACCINES

Between 2002 and 2003, HIV-1-seropositive patients aged 18 – 65 years from the Operative Unit of Infectious Diseases at the Hospital of Gallipoli in Maglie, Lecce, Italy, and the Division of Infectious Diseases of the A Perrino Hospital in Brindisi, Italy, were enrolled in this randomized, open, controlled and comparative study. Detailed clinical and vaccination histories were obtained and physical examinations were performed. Patients with acute febrile disease (> 38 °C), allergy to egg proteins or severe atopy, and those who had previously received an influenza vaccine during the season under study, or who were currently taking part in another experimental clinical study, were excluded from the trial.

The subjects were randomly assigned, in a ratio of 1:1, to receive a single intramuscular dose of influenza MF59-adjuvanted vaccine (Fluad®, Chiron, Siena, Italy) (group A) or subunit vaccine (Agrippal® S1, Chiron) (group B) between November and December 2002. The antigenic composition of these trivalent and inactivated influenza vaccines was that recommended by the WHO for the north-west hemisphere during that season and accepted by the Ministry of Health, Italy. Each vaccine contained 15 µg of superficial haemagglutin antigen for each of the following strains of influenza virus: A/Moscow/10/99 (H3N2), A/New Caledonia/20/99 (H1N1) and B/Hong Kong/330/2001.

Venous blood samples were collected at the time of vaccination (T_0) and then 30 (T_{30}) and 180 (T_{180}) days after administration of the vaccine in order to assess immunogenicity and to evaluate the CD4+ T-lymphocyte count and level of viraemia (HIV-1 RNA).

This study was carried out in accordance with the guidelines of Good Clinical Practices (GCP) and with the approval of the local ethics committee. Written informed consent was obtained from all study participants.

TOLERABILITY AND SAFETY

Each patient was kept under direct observation by the clinical investigator for at least

45 min following vaccination. At the time of the physical examination, each patient received a clinical diary to record the onset, severity and duration of any adverse events, either local or systemic, on the 4 days after immunization. Each patient was also invited to contact the physician using a free-phone service concerning the onset of any serious adverse events (SAEs) during the 30 days after vaccination. When the second blood sample was taken after 30 days (T_{30}), the vaccination diary was collected and a detailed post-vaccination case history obtained, aimed at identifying any adverse events that may have occurred.

CD4+ T-LYMPHOCYTE COUNT

All blood samples were collected in vacutainers containing sodium ethylene diamine tetra-acetic acid. The absolute number of CD4+ T-lymphocytes was determined by means of cytofluorimetric assay (Coulter® Epics XL™/XL-MCL™, Beckman Coulter, Inc., Fullerton, CA, USA) using a commercial kit (Cyto-stat Tetra Chrome, Beckman-Coulter, Inc.). Data were analysed using Tetra One System software (Beckman-Coulter, Inc.).

Patients were subdivided into three categories according to the US Centers for Disease Control and Prevention (CDC) classification on the basis of baseline CD4+ T-lymphocyte count per mm^3 (category 1, $\geq 500/mm^3$, no immunodepression; category 2, $200 - 499/mm^3$, mild immunodepression; category 3, $< 200/mm^3$, severe immunodepression).[17]

PLASMA LEVELS OF HIV-1 RNA

Plasma HIV-1 RNA levels were assessed by quantitative reverse transcription-polymerase chain reaction using a commercial kit with a sensitivity level of 40 copies/ml (Cobas Amplicor HIV-1 Monitor, Roche Diagnostics, Indianapolis, IN, USA).

IMMUNOGENICITY

All sera were analysed for anti-haemagglutin antibodies to the vaccine strains using the haemagglutin inhibition test.[18] The antibody response was evaluated according to the parameters set out by the European Medicines Evaluation Agency (EMEA) for subjects aged between 18 and 60 years and comprising a geometric mean titre (GMT) T_{30}/T_0 ratio ≥ 2.5, seroconversion $\geq 40\%$, and post-vaccine seroprotection $\geq 70\%$.[19] Seroconversion was defined as the onset of a titre in negative subjects or an increase of at least four-fold in the antibody titre between T_0 and T_{30}. Seroprotection was defined as an antibody titre $\geq 1:40$.

STATISTICAL ANALYSIS

Student's *t*-test, χ^2 test, Fisher's exact test and analysis of variance were used in the comparison of immunological parameters, HIV-1 RNA levels and CD4+ T-lymphocyte counts pre- and post-vaccination; data were expressed as means or percentages. For the analysis of viraemia, a value of 39 copies/ml was arbitrarily assigned to samples with values below the dosage cut-off of the kit (< 40 copies/ml). Data were analysed using the STATVIEW 5.0 software (SAS Institute Inc., Cary, North Carolina, USA). A P-value < 0.05 was considered to be statistically significant.

Results

STUDY POPULATION

A total of 40 HIV-1-seropositive patients were enrolled, of whom 37 completed the study. Three patients failed to present for follow-up visits and were therefore considered to have dropped out. Of the remaining 37 subjects, 18 received the adjuvanted vaccine (group A) and 19 received the conventional subunit product (group B). Demographic and clinical baseline data for all the study participants are given in Table 1; there were no

significant differences between the two groups. Most of the patients were receiving highly active antiretroviral therapy (HAART), using different drug protocols.

None of the patients enrolled in the study presented with an influenza-like illness during the follow-up period (from November 2002 to the end of April 2003).

TOLERABILITY AND SAFETY

No SAEs associated with the vaccination occurred during the follow-up period. Onset of at least one local and/or systemic adverse event was recorded in six patients (33%) in group A and in four patients (21%) in group B. Pain and redness at the injection site were the most common reactions (five patients in group A and two in group B), whereas fever occurred in only one and two patients in group A and B, respectively. These events, all considered likely to be vaccination-related, resolved completely within 48 – 72 h.

CD4+ T-LYMPHOCYTE COUNT

Mean lymphocyte counts before vaccination (T_0), at 30 days (T_{30}) and 180 days (T_{180}) after immunization, divided according to the classification categories defined by the CDC, are shown in Table 2. Between-group comparisons of the lymphocyte counts at different observation times (T_0 versus T_{30} and T_0 versus T_{180}) failed to reveal any statistically significant changes, with the exception of subjects with lymphocyte counts < 200 cells/mm^3, vaccinated with conventional subunit vaccine, whose counts showed a progressive increase after immunization (P = 0.048).

TABLE 1:
Demographic and clinical baseline data for 37 human immunodeficiency virus (HIV)-1-seropositive patients before being vaccinated (T_0) against influenza with adjuvanted vaccine (group A) or conventional subunit vaccine (group B)

	Group A	Group B	*P*-value
Total no. of patients	18	19	
Gender			
Male	14 (77.8%)	14 (73.7%)	NS
Female	4 (22.2%)	5 (26.3%)	
Age (years)	40.2 (35.5, 44.9)	37.1 (34.5, 39.7)	NS
CDC category[17]			NS
1	5 (27.8%)	7 (36.8%)	
2	10 (55.6%)	6 (31.6%)	
3	3 (16.7%)	6 (31.6%)	
No. of patients on HAART treatment	15 (83.3%)	15 (78.9%)	NS
CD4+ T-lymphocyte count at T_0	419.7 (294.6, 544.8)	458.9 (404.2, 693.6)	NS
HIV-1 RNA level at T_0 ($\log_{10}$)	2.26 (1.67, 2.85)	3.10 (2.44, 3.76)	NS
No. of patients who received influenza vaccination before 2002	2 (11.1%)	5 (26.3%)	NS

Values are numbers of patients (with percentage in parentheses) or mean value (with 95% confidence interval in parentheses).
CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral therapy; NS, not significant.

TABLE 2:
Mean number of CD4+ T-lymphocytes at the time of vaccination (T_0) and then 30 (T_{30}) and 180 (T_{180}) days after administration of the vaccine in human immunodeficiency virus-1-seropositive patients vaccinated against influenza with adjuvanted vaccine (group A) or conventional subunit vaccine (group B), classified according to baseline cell count

		T_0		T_{30}		T_{180}		
CD4+ T-lymphocyte count	**Group**	***n***	**95% CI**	***n***	**95% CI**	***n***	**95% CI**	***P*-value***
≥ 500 cells/mm^3	A	773	550, 996	735	480, 990	696	539, 853	NS
	B	784	609, 959	960	734, 1186	819	609, 1029	NS
499 – 200 cells/mm^3	A	326	288, 364	408	274, 542	327	287, 367	NS
	B	436	392, 480	397	304, 490	521	469, 573	NS
< 200 cells/mm^3	A	144	55, 233	122	51, 193	134	15, 253	NS
	B	103	73, 133	137	91, 183	223	116, 330	0.048
Total	A	420	295, 545	454	312, 596	406	298, 514	NS
	B	459	314, 604	522	339, 705	541	400, 682	NS

**P*-values were obtained following comparison of data collected at T_0 versus data measured at T_{30} and, subsequently, versus data measured at T_{180}. 95% CI, 95% confidence interval; NS, not significant.

PLASMA LEVELS OF HIV-1 RNA

Viraemia, expressed as the number of RNA copies/ml on a $\log_{10}$ scale, showed a slight increase in patients vaccinated with the adjuvanted product (group A) at all three observation times (Fig. 1). In contrast, patients immunized with the subunit vaccine (group B) showed a slight decrease from T_0 to T_{30} and a slight increase at T_{180}. These changes did not, however, reach statistical significance.

There were no statistically significant inter-group differences in the levels of viraemia at T_0, T_{30} or T_{180} (Fig. 1).

IMMUNOGENICITY

The immunogenicity of the vaccines, classified according to influenza strain and patient group, is given in Table 3. All the parameters recommended by the EMEA were evaluated. In particular, the GMT post-/pre-vaccination (T_{30}/T_0) ratio was always ≥ 2.5 in both groups for all the vaccine strains. Seroconversion parameters were also good, with percentage values ≥ 40% in both the groups under study. No statistically significant differences were found when comparing these intergroup percentages according to viral strain.

Seroprotection parameters, assayed at T_{30} and T_{180}, showed good levels of immunogenicity for both products. The percentage of patients who developed a protective antibody titre (≥ 1:40) within 30 days of vaccination was ≥ 70% in both groups, and remained high even after 180 days.

Discussion

In Italy, influenza vaccination is recommended in all high-risk subjects, including HIV-1-seropositive patients,[20] but the percentage of those in this particular category who are actually immunized is far from

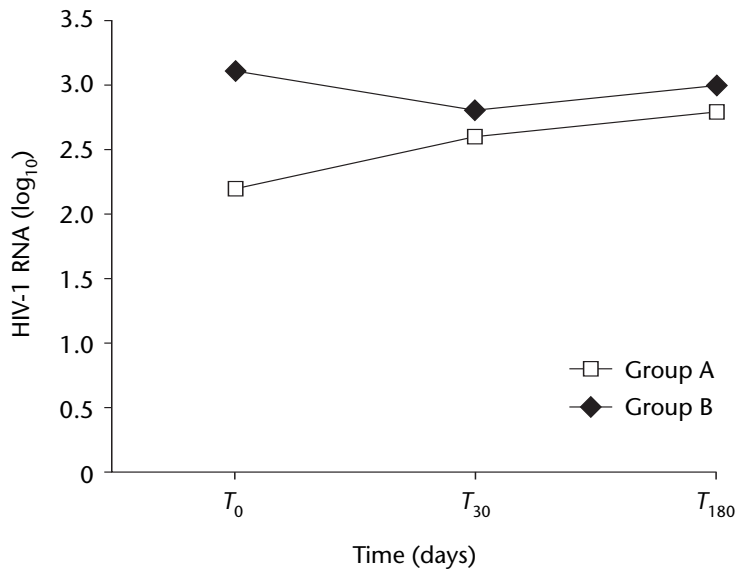

FIGURE 1: Plasma levels of human immunodeficiency virus (HIV)-1 RNA at time of vaccination (T_0) and 30 (T_{30}) and 180 (T_{180}) days after vaccination against influenza with adjuvanted vaccine (group A) or conventional subunit vaccine (group B) in HIV-1-seropositive patients

TABLE 3:
Antibody response (measured by haemagglutin inhibition test) to three different influenza strains in human immunodeficiency virus-1-seropositive patients vaccinated against influenza with adjuvanted vaccine (group A, *n* = 18) or conventional subunit vaccine (group B, *n* = 19)

	A/Moscow/10/99 (H3N2)		A/New Caledonia/20/99 (H1N1)		B/HongKong/330/2001	
	Group A	Group B	Group A	Group B	Group A	Group B
GMT at T_0	17.7	20.0	17.8	37.0	24.2	34.3
GMT at T_{30}	56.6	83.0	89.8	214.2	113.1	178.5
GMT T_{30}/T_0 ratio	3.2	4.1	5.0	5.8	4.7	5.2
Seroconversion	8 (44%)	10 (53%)	11 (61%)	13 (68%)	13 (72%)	10 (53%)
Seroprotection						
At T_0	3 (17%)	6 (32%)	5 (28%)	11 (58%)	7 (39%)	9 (47%)
At T_{30}	13 (72%)	14 (74%)	15 (83%)	19 (100%)	17 (94%)	17 (89%)
At T_{180}	10 (55%)	9 (47%)	9 (50%)	14 (74%)	9 (50%)	11 (58%)

Seroconversion was defined as the onset of a titre in negative subjects or an increase of at least four-fold in the antibody titre between T_0 and T_{30}.
Seroprotection was defined as an antibody titre ≥ 1:40.
GMT, geometric mean titre; T_0, at the time of vaccination; T_{30}, 30 days after vaccination; T_{180}, 180 days after vaccination.

satisfactory. Similar discrepancies have been reported in several studies. This situation may arise due to a combination of factors, including lack of adequate information for patients regarding the real risks of influenza, and doubts concerning the safety and efficacy of the vaccine in immunodepressed subjects.[21,22] In the present study, the majority of the enrolled HIV-1-seropositive patients (30 of 37, 81%) reported not having routinely received influenza vaccination (Table 1).

Several studies on HIV-1-seropositive subjects have demonstrated that influenza vaccination is safe and well tolerated, both in adults and children; the incidence of SAEs is rare and the incidence of local and systemic side-effects is comparable to that in the general population.[9,23 – 27]

The increase in viraemia and CD4+ T-lymphocyte depletion following vaccination reported by some authors has contributed to the limited use of influenza vaccine, due to the hypothetical negative effect on the progression of HIV-related disease.[13,28 – 30] The increase observed in viraemia levels generally reached a peak 2 – 3 weeks after immunization, and the frequency and severity of this phenomenon seemed to be strictly related to the basal levels of viraemia. It also appeared more liable to occur in patients who were likely to develop an adequate immune response to the vaccination.[24] In contrast, other studies have shown that the increase in viraemia levels following vaccination was transient and of no clinical relevance; moreover, these studies did not demonstrate significant changes in the plasma levels of CD4+ T-lymphocytes.[31 – 33]

In the present study, no SAEs occurred following vaccination with either of the products used, and all side-effects, both local and systemic, disappeared within 48 – 72 h; these results are in agreement with many other findings reported in the literature. In particular, no significant changes of clinical importance were observed when comparing viraemia levels and CD4+ T-lymphocyte counts before vaccination, and 30 days and 180 days after vaccination. This appears to confirm that vaccination does not affect these parameters in any way; the hypothesis of an increased risk of a rise in viral load in HIV-1-seropositive subjects immunized with an adjuvanted vaccine as a result of increased antigenic stimulation was not supported by the present study. These findings demonstrate the excellent safety profile of the MF59-adjuvanted influenza vaccine, already described by Iorio *et al.*[12] in a similar study, and thus confirm the suitability of influenza vaccination for HIV-1-seropositive immunodepressed subjects. HIV-1-RNA levels and CD4+ T-lymphocyte counts were not monitored weekly during the month following vaccination, as has been done by others,[14,31] since the aim of this study was to look exclusively for clinically significant changes rather than to study viral kinetics.

Data from the present study also demonstrate that CDC classification or the concurrent use of antiviral pharmacological protocols do not appear to influence the effect of vaccination on viraemia levels or lymphocyte count, confirming the absence of any negative effect of the influenza vaccine in HIV-1-infected individuals.

There is clear evidence that HIV-1 infection affects the onset of the antibody response to influenza vaccine, particularly in patients with advanced disease and severe immunodeficiency.[24] Several immunogenicity studies have demonstrated that HIV-1-seropositive patients present a lower immune response than non-infected subjects,[31,34 – 37] and that the immunological memory induced by immunization seems to be weak, especially in patients with

advanced disease.[24] The ability of specific HAART to induce an increase in the number of CD4+ T-lymphocytes and an improvement in function seems to be associated with an improved antibody response in these patients.[38]

In the present study, data on immunogenicity were optimal with both the vaccines used; all the parameters were in keeping with those set out by the EMEA. Another reassuring aspect, observed in both groups, was the prolonged persistence of specific antibodies towards all the vaccine strains, even 180 days after vaccination (T_{180}): this was shown by the percentage of patients with seroprotection. This finding is, at least in part, due to the good immunological and general status of the patients enrolled in the study. Most patients were following a HAART pharmacological protocol under the strict surveillance of a specialist in infectious diseases, and only nine of the 37 patients (24.3%) belonged to CDC category 3 (i.e. severe immunodeficiency), who are unanimously recognized as hypo-responders to vaccination.

In conclusion, the results of the present study confirm the safety and immunogenicity of these currently available vaccines in HIV-1-seropositive patients, and thus support the recommendation for influenza immunization in this high-risk category. The limited number of subjects enrolled means it was not possible to draw definitive conclusions regarding the type of vaccine used.

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Conflicts of interest

No conflicts of interest were declared in relation to this article.

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