Acta virologica **56**: 349 – 351, 2012 doi:10.4149/av_2012_04_349

LETTER TO THE EDITOR

Immunogenicity and safety of pandemic H1N1 2009 influenza vaccine for HIV-1 patients

D. JILICH[1], M. HAVLÍČKOVÁ[2], D. VESELÝ[3], H. ROZSYPAL[1], H. JIŘINCOVÁ[2], M. STAŇKOVÁ[1], J. KYNČL[2], M. MALÝ[4], L. MACHALA[5*]

[1]Department of Infectious and Tropical Diseases, First Faculty of Medicine, Charles University in Prague and Na Bulovce Hospital, Prague, Czech Republic; [2]National Reference Laboratory for Influenza, National Institute of Public Health, Prague, Czech Republic; [3]Department of Infectious and Tropical Diseases, Na Bulovce Hospital, Prague, Czech Republic; [4]Department of Biostatistics and Informatics, National Institute of Public Health, Prague, Czech Republic; [5]Department of Infectious, Parasitic and Tropic Diseases of the Third Faculty of Medicine and Na Bulovce Hospital, Prague, Czech Republic

Received May 28, 2012; accepted October 22, 2012

Keywords: HIV-1 patients; pandemic influenza H1N1; vaccination

Influenza is one of the most common infectious diseases, and human immunodeficiency virus 1 (HIV-1)-infected individuals are particularly endangered by development of its complications (*1*). Influenza is, however, effectively preventable by annual vaccination, and regular vaccination is recommended for both the general population and all endangered groups. Vaccination against seasonal influenza has also been proven to be effective and safe in several studies with HIV-1-infected subjects. For safety reasons, HIV-1-infected individuals are vaccinated only with inactivated influenza vaccines. HIV-1-infected individuals with CD4+ T lymphocyte (CD4+) counts >500 cells/μl are generally regarded as sufficiently immunocompetent, whereas those individuals with CD4+ counts < 200 cells/μl are considered to have significantly reduced immunoresponsiveness (*2*). Titers of post-vaccination hemagglutinin-specific antibodies ≥40 are generally considered protective and, according to the requirements of the Committee for Medicinal Products of the European Medicines Agency (EMA/CHMP), the protection rate should reach at least 70% of vaccinees, the response rate – defined as a 4-fold or higher increase in HA-specific antibody titers – should reach a minimum of 40% vaccinees, and the conversion factor – defined as the ratio of the post-vaccination geometric mean titers to the pre-vaccination geometric mean titers – should exceed 2.5 (*3*).

The advent of the 2009 H1N1 pandemic influenza A virus has raised concerns about the immunogenicity and safety of newly developed vaccines, especially in immunocompromised individuals.

In our study, we evaluated the immunogenicity and safety of a new adjuvanted pandemic influenza vaccine in 34 HIV-1-infected patients: 33 males and 1 female at the mean age of 43 years (range 27–71). All vaccinees were over the age of 18 years and signed the informed consent form. None of them had received influenza vaccinations in the three seasons prior or suffered from an episode of a febrile illness during the last six months. The mean baseline CD4+ count was 568 cells/μl (range 90–1151) and the mean nadir CD4+ count was 306 cells/μl (range 6–673). Twenty-six patients (76.5%) were on combination antiretroviral therapy and 23 (88.5%) of them had a baseline HIV RNA viral load of < 20 copies/ml. In five subjects (11.2%), HA-specific antibodies were already found in the pre-vaccination serum: three of them had low

*Corresponding author. E-mail: ladimachala@centrum.cz; phone: +4202-66082629.

Abbreviations: CD4+ = CD4+ T lymphocyte; EMA/CHMP = Committee for Medicinal Products of the European Medicines Agency; HA = hemagglutinin, HIV = human immunodeficiency virus

Table. Characteristics of vaccinees with and without protective (≥40) post-vaccination anti-HA titers

	Seroprotection (n = 24)	No seroprotection (n = 10)	p-value
Mean age in years (standard deviation)	44.1 (9.5)	40.9 (12.3)	0.411
Mean baseline CD4+ cells/μl (standard deviation)	604 (276)	480 (261)	0.232
Mean nadir CD4+ cells/μl (standard deviation)	318 (161)	276 (192)	0.511
Mean duration of HIV infection in years (standard deviation)	8.6 (6.1)	8.6 (6.8)	0.976
Number of vaccines with a baseline viral load of <20 HIV RNA copies/ml (%)	19 (79.2%)	3 (30.0%)	0.015
Number of vaccines on combination antiretroviral therapy (%)	20 (83.3%)	6 (60.0%)	0.195
Geometric mean of baseline titers (95% CI)	1.6 (0.9-2.3)	1.3 (0.4-2.1)	0.532
Geometric mean of post-vaccination titers (95% CI)	84.8 (45.5-124.0)	6.0 (1.7-10.4)	<0.001

titers of 10, and the other two subjects had titers of 20 and 40, respectively.

Inactivated adjuvanted split H1N1 influenza vaccine (Pandemrix®, GlaxoSmithKline), containing 3.75 μg of hemagglutinin (HA) A/California/7/2009 and the adjuvant AS03, was used for vaccination. Two serum samples were collected from the vaccinees: the first sample on day 0 at the time of vaccination, and the second on day 28 ± 2 after the vaccination. The vaccinees were also asked to note possible reactions and/or side effects. Antibody production was tested by hemagglutination-inhibition assay according to WHO methodology (*4*). The levels of protective antibodies were quantified and evaluated according to the standard parameters set by the EMA/CHMP (*3*).

Arithmetic means, standard deviation, 95% confidence intervals, counts and proportions were calculated for statistical analysis. Unadjusted group comparisons are based on a two-sided, two-sample Student's t-test and Fisher's exact test. Potential predictors for reaching protective titers were assessed by logistic regression.

Twenty-eight days after the vaccination, protective anti-HA titers ≥40 were found in 24 (70.6%) vaccinees with a mean baseline CD4+ count of 604 cells/μl and a nadir CD4+ count of 318 cells/μl. The response rate was 79.4% (27 vaccinees) and the conversion factor was 26.1. Post-vaccination anti-HA titers ≥40 were not found in ten (29.4%) individuals with a mean baseline CD4+ count of 480 cells/μl and a nadir CD4+ count of 276 cells/μl. These CD4+ counts were lower than in the vaccinees with protective titers, but the difference was not significant. Four vaccinees (11.2%) had undetectable anti-HA titers in both the baseline and post-vaccination serum samples. Their mean nadir CD4+ count was 309 cells/μl (range 79–400) and their mean baseline CD4+ count was 724 cells/μl (range 342–1151).

In a logistic regression model with an indicator of protective titers as the dependent variable, only the viral load was significantly associated (odds ratio = 0.06, p = 0.022) with the outcome, whereas the time from HIV diagnosis, age, and CD4+ counts remained insignificant. The basic characteristics of the vaccinees with and without protective post-vaccination antibody titers are summarized in the Table. No serious adverse events were observed; only nine individuals (26.5%) reported moderate pain at the application site lasting a maximum of 2 days.

In our study, 24 (70.6%) vaccinees had protective anti-HA titers 28 days after the vaccination. In the studies with non-adjuvanted monovalent pandemic vaccines with 15 μg of HA, the protection rate ranged from 60.8% to 75.5% (*5*, *6*). In the studies with adjuvanted vaccines (AS03 + 3.75 μg HA), the protection rate ranged from 45.2% to 92.2% (*7*, *8*). In the studies with another adjuvanted vaccine (MF59 + 7.5 μg of HA), the protection rate was between 78% and 97.7% (*9*, *10*).

The protection rate of 70.6% reached in our cohort thus exceeded, albeit only narrowly, the limit required for the normal population. Other parameters, such as the 26.1 conversion factor and the 79.4% response rate, also exceeded the requirements of the EMA/CHMP (*3*).

The reasons why ten (29.4%) vaccinees did not produce protective titers despite relatively high CD4+ counts are not clear. A sufficient number of CD4+ cells, is generally considered an important factor for humoral immune response; however, in several other studies with pandemic vaccines, the predictive value of the CD4+ count was also not found (*5*, *7*, *11*). A possible explanation for reduced immunogenicity of vaccination in individuals without serious immunodeficiency may be found in immune dysregulation affecting T- and B-cell quantities and functions, immune activation or immunosenescene (*12*). In one study (*13*), the production of protective antibodies correlated with an increase in IL21 levels and IL21-R-expressing B-cells. Unfortunately, these markers were not tested in any other published studies.

Similarly to other studies, our study did not confirm the predictive values of factors such as age, baseline HIV suppression, or antiretroviral use (*5*, *7*, *11*, *14*). Of course, the major limitation in assessing the predictive value of those

factors may likely be attributed to the relatively low number of subjects tested in most of the published studies.

Five of our vaccinees had baseline titers of HA-specific antibodies. The very low titers of ≤10 that were found in three patients can occur despite preparation of the serum and removal of non-specific inhibitors and do not imply previous exposure to the pandemic 2009 virus (*15*). The baseline HA-specific antibody titers of 20 and 40 detected in two other individuals do not exclude the possibility of previous infection with the pandemic 2009 virus. None of the participants experienced any acute respiratory infection in the 6-month period preceding the vaccination. However, since the presence of the pandemic 2009 virus was confirmed in the Czech Republic six months before the start of the study, exposure to the virus resulting in asymptomatic infection may not be excluded.

The only side effect observed in our study was moderate pain at the application site with an incidence of 26.5%. This occurrence is within the range of incidence reported in other studies, varying between 1.8% in one study where a non-adjuvanted pandemic vaccine was used (*14*) and 84% observed in another study where the AS03 adjuvanted vaccine was used (*7*).

The production of protective antibodies after vaccination of HIV-1-infected individuals with a single dose of inactivated pandemic adjuvanted influenza vaccine exceeded beyond the limits set by EMA/CHMP for the healthy adult population (*3*). The results of our study thus confirmed satisfactory immunogenicity and good tolerability of the tested vaccine and helped to reduce the concerns about the inferiority of vaccines based on novel pandemic influenza A virus antigens.

Acknowledgement. This work was supported by the grant No. SVV-2012-264506 from the Charles University of Prague, Czech Republic.

References

1. Sheth A, Althoff K, Brooks J, Clin. Infect. Dis. 52, 219–227, 2011. http://dx.doi.org/10.1093/cid/ciq110

2. Anema A, Mills E, Montaner J, Brownstein J, Cooper C, HIV Med. 9, 57–61, 2008. http://dx.doi.org/10.1111/j.1468-1293.2008.00515.x

3. EMEA/CHMP/VWP: Guideline on Clinical Evaluation of New Vaccines. 2006.

4. WHO Manual on Animal Influenza Diagnosis and Surveillance: www.who.int, 2002.

5. Launay O, Desaint C, Durier C, Loulergue P, Duval X, Jacomet C, Pialoux G, Ghosn J, Raffi F, Rey D, Ajana F, de Verdière NC, Reynes J, Foubert V, Roman F, Devaster J-M, Delfraissy J-F, Aboulker J-P, J. Infect. Dis. 204, 124–134, 2011. http://dx.doi.org/10.1093/infdis/jir211

6. Tebas P, Frank I, Lewis M, Toma E, Sylla M, Cyr L, Cote S, Baz M, Sampalis J, Trautman L, Sekaly R, Boivin G, AIDS 24, 2187–2192, 2010. http://dx.doi.org/10.1097/QAD.0b013e32833c6d5c

7. Tremblay C, Rouleau D, Fortin C, Toma E, Sylla M, Cyr L, Cote S, Baz M, Sampalis J, Trautman L, Sekaly R, Boivin G, Vaccine 29, 1359–1363, 2011. http://dx.doi.org/10.1016/j.vaccine.2010.12.023

8. Canestri A, Krivine A, Assoumou L, Le Corre M, Rozenberg F, Marcelin A, Schneider L, Samri A, Carcelain G, Autran B, Katlama C, Guihot A, AIDS 24, 2887–2889, 2010. http://dx.doi.org/10.1097/QAD.0b013e3283402bc1

9. Fabbiani M, Di Giambenedetto S, Sali M, Farina S, Sansonetti P, Tamburrini E, Dal Verme L, Delogu G, De Luca A, Kelvin D, Cauda R, Fadda G, Vaccine 29, 2836–2839, 2011. http://dx.doi.org/10.1016/j.vaccine.2011.02.020

10. Kajaste-Rudnitski A, Galli L, Nozza S, Tambussi G, Di Pietro A, Pellicciotta G, Monti A, Mascagni P, Moro M, Vicenzi E, AIDS 25, 177–183, 2011. http://dx.doi.org/10.1097/QAD.0b013e328341afa8

11. Crum-Cianflone N, Eberly L, Duplessis C, Maguire J, Ganesan A, Faix D, Defang G, Bai Y, Iverson E, Lalani T, Whitman T, Blair P, Brandt C, Macalino G, Burgess T, Clin. Infect. Dis. 52, 138–146, 2011. http://dx.doi.org/10.1093/cid/ciq019

12. Molina-Pinelo S, Vallejo A, Diaz L, Soriano-Sarabia N, Ferrando-Martinez S, Resino S, Munoz-Fernandez M, Leal M, J. Antimicrob. Chemother. 2009; 64, 579–588, 2009. http://dx.doi.org/10.1093/jac/dkp248

13. Pallikkuth S, Pilakka Kanthikeel S, Silva S, Fischl M, Pahwa R, Pahwa S, J. Immunol. 186, 6173–6181, 2011. http://dx.doi.org/10.4049/jimmunol.1100264

14. Yanagisawa N, Maeda K, Ajisawa A, Imamura A, Suganuma A, Ando M, Takayama N, Okuno Y, Vaccine 29, 5694–5698, 2011. http://dx.doi.org/10.1016/j.vaccine.2011.06.003

15. Papenburg J, Baz M, Hamelin M, Rheaume C, Carbonneau J, Ouakki M, Rouleau I, De Serres G, Boivin G, Clin. Vaccine Immunol. 18, 520–522, 2011. http://dx.doi.org/10.1128/CVI.00449-10