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A randomized clinical trial assessing immunogenicity and safety of a double dose of virosomal-adjuvanted influenza vaccine administered to unprimed children aged 6–35 months

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ABSTRACT

This study evaluated the immunogenicity of a double dose of the seasonal virosomal-adjuvanted influenza vaccine (Inflexal V, Crucell, The Netherlands) in 65 previously unvaccinated children aged less than 3 years: 43 received double doses (two doses of 0.50 mL 4 weeks apart) and 22 standard doses (two doses of 0.25 mL 4 weeks apart). Both treatments evoked a response that satisfied the EMEA criteria for adequate immunogenicity for all three vaccine strains, but the double dose had a significantly greater effect on all of the studied parameters of humoral and cell-mediated immune response ($p < 0.05$). This result was achieved without any increase in the incidence of local and systemic adverse events. This means that doubling the dose of the virosomal-adjuvanted influenza vaccine (i.e. administering the same dose as that usually given to older children) effectively and safely increases the immune response to inactivated influenza vaccine in unprimed children aged less than 3 years.

1. Introduction

Because seasonal influenza can be dangerous for children with an underlying chronic severe disease, health authorities throughout the world have strongly recommended that they be administered influenza vaccination [1–3]. A number of recent studies have clearly shown that seasonal influenza can also have a negative medical and socioeconomic impact on healthy infants and children, particularly those aged less than 5 years [4–7]. Consequently, in some countries such as the United States and Finland, seasonal influenza vaccine is now also recommended for healthy children [8,9]. However, most (particularly European) health authorities do not agree, mainly because the immunogenicity and efficacy of conventional trivalent inactivated vaccines in younger subjects are considered to be too low to justify its universal use [10]. On the other hand, live attenuated influenza vaccine, which has been demonstrated to be more immunogenic and effective than inactivated vaccines [11], is not available in Europe and, in North America (where it is marketed), it is only licensed for children aged more than 2 years and cannot be administered in children with asthma and recurrent wheezing [12].

A number of measures have been explored to increase immune response to seasonal influenza inactivated vaccines in adults and elderly subjects, and multiple administrations, intradermal injections, adjuvanted vaccines, and vaccines containing an increased dose of antigens have all been tested with varying results [13–21]. However, there are few data concerning attempts to increase such responses in children. Multiple administrations cannot be suggested because of their already crowded immunisation schedule, particularly in the first years of life, and in any case cannot be used in unprimed children for whom two injections of influenza vaccine 1 month apart have to be administered. Although intradermal injections assure a better immune response than intramuscular or subcutaneous injections, there is a lack of data regarding the exact amount of antigens needed to obtain a significant and protective increase in antibody production [22,23]. Furthermore, in order to be easy-to-perform, reliable and safe, intradermal vaccine administration requires special microinjection systems that increase vaccination costs.

The data concerning adjuvants seem to suggest that the two adjuvanted vaccines that have been evaluated in younger children can be effective in increasing the immune response to seasonal

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influenza antigens. However, when MF59-adjuvanted vaccine was used, the significant increase in the immune response of unprimed children to all of the viral antigens included in the vaccine was accompanied by a greater number of solicited reactions [24]. Furthermore, although the administration of virosomal-adjuvanted vaccine was not followed by an increase in the number of adverse events, the increase in seroprotection was statistically significant only for the H3N2 antigen [25,26]. Finally, there are no published pediatric data regarding the administration of an increase amount of seasonal influenza antigens. Only data regarding a monovalent pandemic vaccine administered with different dosages are available [27]. However, conclusions derived from this study cannot be transferred to seasonal vaccination for two reasons. Firstly, the hemagglutinin of the pandemic virus seems to be more immunogenic than those of seasonal strains evoking in children immune response consistent with that observed in adults [28]. Secondly, doses of antigens significantly higher than that usually employed in the seasonal vaccination of unprimed children were administered. This means that further studies are needed to establish the best way to increase seasonal influenza vaccine immunogenicity in unprimed children without increasing the number or severity of adverse events. The aim of this study was to explore this problem comparing the immunogenicity and the safety of double and standard doses of a seasonal virosomal-adjuvanted influenza vaccine in previously unvaccinated children aged less than 3 years.

2. Patients and methods

2.1. Study design and population

This prospective, randomized, partially blinded study compared the immunogenicity and safety of standard doses of the virosomal-adjuvanted influenza vaccine with those of double doses in healthy children aged 6–35 months who had not been previously vaccinated against influenza. It was carried out in the outpatient clinic of the Department of Maternal and Pediatric Sciences of the University of Milan, Italy, between 1 October 2008 and 31 May 2009. Influenza vaccination was offered to all the children aged 6–36 months of age admitted to the outpatient clinic for a control visit after a previous hospitalization for minor surgical problems. The exclusion criteria were any previous influenza vaccination, chronic disease, a known allergy to any vaccine component, any acute infectious or respiratory disease requiring systemic treatment in the 30 days preceding the start of the study, or rapid-test or laboratory-confirmed influenza in the previous 6 months.

The study was approved by the Ethics Committee of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, and was conducted in accordance with the standards of Good Clinical Practice for trials of medicinal products in humans. Written informed consent was obtained from the parents/legal guardians of each enrolled child. Designated, unblinded study staff (SB and EB) administered the vaccine on the basis of a computer-generated randomisation list and had no further contact with the subjects or access to the data. All the other pediatricians involved in the study, as well as the enrolled families, were blinded throughout the study.

The children were randomised 1:2 to receive intramuscularly in the lateral upper thigh two doses of 0.25 mL (standard dose) or 0.50 mL (double dose) of the 2008–2009 seasonal virosomal-adjuvanted influenza vaccine (Inflexal V, Crucell, The Netherlands) separated by an interval of 4 weeks. Sequence of assignment was generated with a computer randomization by a statistician who was not involved in the rest of the trial. Assignments were enclosed in sequentially numbered, identical, sealed envelopes. Masking was maintained since all vaccinations were done by specific study personnel, who did not take part in the assessment of safety or immunogenicity. Each dose of the vaccine contained 7.5 or 15 μg each of the A/Brisbane/59/2007/H1N1-like, A/Brisbane/10/2007/H3N2-like and B/Florida/4/2006-like purified influenza surface antigens hemagglutinin integrated into the lipid membrane of the virosome, with solvent added to reach a volume of 0.25 or 0.50 mL.

Immunogenicity assessments were made before the first vaccination dose on day 1 (baseline), on day 28 ± 3 (4 weeks after the first vaccine dose), on day 56 ± 3 (4 weeks after the second vaccination), and on day 210 ± 3 (6 months after the second vaccination).

Safety was assessed in all the subjects who had received at least one dose of vaccine and for whom post-baseline safety data concerning local and systemic reactions were available. Local and systemic reactions were assessed by the investigators at baseline and during the follow-up visits, and by the children's parents/legal guardians for 14 days after each vaccination. The children were examined for the presence of local adverse events (AEs; pain/tenderness, redness and swelling/induration) and questioned about systemic AEs (body temperature >38 °C, malaise, irritability, vomiting, cough).

2.2. Assessment of humoral immune response

Haemagglutination-inhibiting (HI) antibodies were titred, as previously described [18,29,30] against each of the three influenza strains in the 2008–2009 formulation in all the children who received all the doses of the vaccine correctly, provided evaluable serum samples at all scheduled time points, and had no major protocol violations. The HI antibody titre was expressed as the reciprocal of the highest dilution inhibiting agglutination. As previously described [31], in order to allow the calculation of the HI geometric mean titres (GMTs), a titre of 1:5 was assigned arbitrarily to non-responders. The immunogenicity endpoints were based on the haemagglutination inhibition licensure criteria established by the guideline of the European Agency for the Evaluation of Medical Products (EMEA) [32]. Immunogenicity was determined by: GMT; mean-fold increase (MFI: ratio of post- to prevaccination titre); seroprotection rate (the percentage of subjects achieving an HI titre $\geq$40); and seroconversion rate (percentage of subjects with a 4-fold increase in antibody titers, providing a minimal post-vaccination titer of 1:40) [32]. As there are no EMEA-defined criteria for children, immunogenicity was evaluated on the basis of the criteria for adults aged 18–60 years, which require at least one of the following for each strain [25]: (1) seroconversion, a $\geq$4-fold increase in HI antibody titre, with a titre of $\geq$1:40 being reached in >40% of the subjects; (2) seroprotection, an HI antibody titre of $\geq$1:40 in >70% of the subjects; and (3) GMT, a >2.5-fold increase in the HI antibody GMT.

2.3. Assessment of cell-mediated immunity

Cell-mediated immunity was assessed on peripheral blood mononuclear cells (PMBCs) derived from blood samples collected during the assessment visits and placed in Vacutainer tubes containing ethylendiaminetetraacetic acid (EDTA, Becton Dickinson, Rutherford, NJ). The PMBCs were separated on lymphocyte separation medium (Organon Teknica, Durhan, NC) and washed twice in phosphate-buffered saline (Organon Teknica). The number of viable leukocytes was determined by trypan blue exclusion. All of the analyses were made using freshly collected cells.

As previously described [33], for the enzyme-linked immunospot (ELISPOT) assays, 96-well nitrocellulose plates were coated with a first layer of interferon (IFN)-γ monoclonal antibody (Pierce Biotechnology, Rockford, IL, USA) for 18 h at 4 °C. A total of 2.5×10^5 PBMCs/well were then added to duplicate wells in the presence of neutralising anti-CD4 monoclonal antibody (R&D