

REVIEW

Immunogenicity and effectiveness of virosomal adjuvanted vaccines against influenza: a brief review of their utility in the elderly population

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Key words

Influenza • Virosomal adjuvanted vaccine • Immunogenicity

Summary

The World Health Organisation (WHO) estimates that seasonal influenza affects approximately 5-15% of the population on an annual basis and these outbreaks are responsible for significant morbidity and mortality resulting in increased hospital admissions. Annual vaccination remains the main public health strategy for containing influenza. Inactivated influenza vaccines offer a great deal of protection against influenza but these vaccines are not optimal for older adults due to their waning immunity and

other factors affecting immunogenicity. Virosomes, stimulate the immune system in a similar way as a natural infection and studies have shown that the virosomal adjuvanted influenza vaccine is immunogenic and safe in different population groups including: the elderly, children and immunocompromised subjects. Currently available data suggest improved immunogenicity of the virosomal adjuvanted vaccine in the elderly and in subjects without protective antibody as compared to conventional vaccines.

Introduction

Each year seasonal influenza outbreaks are responsible for significant morbidity and mortality resulting in increased hospital admissions. The World Health Organisation (WHO) estimates that seasonal influenza affects approximately 5-15% of the population on an annual basis [1]. Between 2000 and 2008, influenza along with other acute upper respiratory infections accounted for about 44 000 hospitalisations per year in France and 77000 in Germany [2]. The elderly and persons with comorbid conditions are at highest risk for complications and death during these epidemics most likely as a result of decreased immune system function [3]. Organisation for Economic Co-operation and Development (OECD) estimates that between 85-90% of people who die from influenza in France and Germany are over 65 years of age. [1]. Annual vaccination remains the main public health strategy for containing influenza. Among the elderly, vaccination against influenza remains a high priority and has been shown to be effective in reducing influenza associated morbidity and mortality. Consequently, in the US and Europe most current vaccination programs recommend vaccination against influenza in older adults.

There are several factors responsible for the vulnerability to influenza seen in the elderly population; the most important is the phenomenon of immunosenescence. Immunosenescence is a physiological condition resulting from advanced age and which is associated with impairments in both innate and adaptive immunity. Consequently, there is a need for adapted vaccines for elderly in order to improve immune responses. For the past 60 years, the so-called "conventional vaccines" (subunit

and split non adjuvanted vaccines) have accounted for the majority of prophylactic vaccines used against influenza [4]. However, these "conventional vaccines" have also shown some limitations in effectiveness especially in the elderly population and populations affected by chronic diseases or immunocompromised. In addition, lack of cross protection against some drifted influenza strains accounts for some loss of effectiveness. In a randomized controlled trial in persons aged ≥ 60 years the efficacy against laboratory-confirmed influenza illness was 58% in a season when the vaccine strains were well-matched to circulating strains [3]. In other studies, performed in 2 following seasons, the vaccine efficacy in the elderly ≥ 65 years was 57.11% (1999-2000 season) and 45.74% (2000-2001 season) respectively [5, 6]. In the search to fill gaps in efficacy of the conventional vaccines and boost the immunogenicity of these vaccines, in the past few years several new vaccine formulations have been investigated. Since 1997, the virosomal adjuvanted vaccine (Inflexal V[®], Crucell, Switzerland), has been used widely in several European countries and has shown good results in terms of tolerability and safety. In addition, immunogenicity is significantly better than seen in "conventional vaccines" [7-10]. This brief review discusses the most recent findings on immunogenicity and effectiveness of virosomal adjuvanted vaccines in the elderly population.

ADJUVANT VACCINES

Adjuvants have been used in vaccinology for decades. Their use has allowed major improvements in the immunogenicity of vaccines with the added benefit of signifi-

cantly reducing the amount of antigen needed to produce an immune response. They work in several ways: they can modify the cytokine network favouring antigen uptake by antigen presenting cells and induction of cytotoxic T lymphocyte responses, they may prolong antigen release and target antigens for presentation by MHC class I or class II molecules thus inducing direct immunity against intracellular and extracellular pathogens [11, 12]. Currently licensed adjuvants can be classified into 3 main categories [13]: mineral salts (aluminium hydroxide, alum), oil emulsions (MF59, AS03 and AF03) and particulate delivery systems (viroosomes, AS04). The virosomal adjuvanted vaccines are the focus of this discussion.

VIROSOMAL ADJUVANTED INFLUENZA VACCINE

Viroosomes, also called Immunopotentiating Reconstituted Influenza Viroosomes (IRIVs), are empty influenza virus particles deprived of an internal nucleus and genetic information. They stimulate the immune system in a similar way as a natural infection. They consist of phospholipids which form a continuous, spherical lipid bilayer with an aqueous inner compartment, which can be loaded with antigens. In addition, hemoagglutinins and neuroaminidases are integrated into the bilayer, as does a natural influenza virus, but IRIVs do not contain viral RNA and viral nucleocapsid proteins [9]. The dimension of the virosome (150 nm) is "appetizing" to Antigen Presenting Cells (APC) [14]. The adjuvant property of IRIVs results from the particulate structure and from the presence of biologically active influenza hemagglutinins in their membrane [9, 12]. The repetition of antigens on their surface sends a strong signal of activation to the B-cells which in turn stimulates production of antibodies [15]. The HA maintains the fusion activity, binding to the APC receptors and mediating the fusion of the virosome with membrane of the APC endosome. The processing of antigen in 2 different T cellular compartments leads to different effects. Antigen processed in the endosome leads to its presentation associated with the Major Histocompatibility Complex II (MHC II) whereas antigen processed in the cytosol leads to its presentation in association with the Major Histocompatibility Complex I (MHC I) [14]. In this way influenza virosomes are able to stimulate both the humoral and the cellular immune pathways [16-18]. The virosomal adjuvanted vaccine does not contain preservatives such as formaldehyde or thiomersal and they do not induce anti-phospholipid antibodies [19, 20]. It has the lowest content of ovalbumin among other egg-cultivated vaccine, has been approved for all age groups and are well tolerated [9, 20, 21].

IMMUNOGENICITY AND EFFECTIVENESS OF INFLUENZA VACCINES

Vaccine *effectiveness* refers to the level of protection that a vaccine can be expected to achieve under ordinary field conditions of a Public Health Program [22]. Effectiveness is affected by the conditions under which the vaccine is used as well as the target population and the efficacy. It is usually measured in observational studies. Vaccine *efficacy* on the other hand is assessed in controlled clinical trials [3].

Immunogenicity refers to the ability of a vaccine to induce an immune response (antibody and/or cell-mediated immunity) in a vaccinated individual [23]. For influenza vaccines a hemagglutination-inhibition antibody titer of 1/40 is considered a correlate of protection [24]. In 1997, the European Medicines Agency (EMA), whose mandate is to evaluate medicines produced by pharmaceutical companies for use in the European Union (EU), established criteria for safety and immunogenicity of vaccines produced for use in the EU. For influenza vaccines to be accepted throughout the EU, annual clinical trials must demonstrate immunogenicity and safety in at least 50 subjects between 18 and 60 years and in 50 subjects over 60 years. Vaccines must fulfil at least one of the three EMEA criteria for each influenza strain contained in the vaccine for both age groups (18-60 and over 60). These criteria are defined as follows: a seroconversion rate (SC, defined as a \geq four-fold increase in HA inhibition antibody titre to a titre \geq 1:40) should be $> 40\%$ for adults and 30% for persons over 60; the proportion of seroprotection (SP, defined as HA inhibition titres \geq 1:40) should be $> 70\%$ for adults and 60% for persons over 60, and the increase in Geometric Mean Titer (GMT) should be > 2.5 fold for adults and 2 fold for persons over 60 [25].

IMMUNOGENICITY AND EFFECTIVENESS OF VIROSOMAL ADJUVANTED VACCINES

Since 1997 the virosomal adjuvanted influenza vaccine has met the EMA criteria in the annual clinical trials. Furthermore studies have been conducted in different groups who can benefit from an adjuvanted vaccine, as elderly, children and immunocompromised subjects. In all these groups the virosomal adjuvanted influenza vaccine has shown to be immunogenic and safe [9].

In children of 6-71 months the SP and SC rates for strain A/H1N1 were significantly higher after 2 doses (0.25 ml or 0.5 ml according to the age group) 4 weeks apart of the virosomal adjuvanted vaccine (SP 88.8% and SC 88.8%) than after the split vaccine (SP 78.3% and SC 77.5%). Both vaccines were well tolerated [26].

In a follow-up study the same authors evaluated the immunogenicity and the cellular immune response of 2 doses of 0.25 ml compared to 2 doses of 0.5 ml of virosomal adjuvanted vaccine in children 6-35 months of age. Both the treatments met the EMA criteria after 2 doses and already after the first dose of 0.5 ml EMA criteria were satisfied, in particular SC and GMT increase were reached for all three influenza strains and SC for 2 strains (A/H3N2 and A/H1N1). Also the cytokines production was significantly higher in the 0.5 ml group, both after the first and the second dose; on the contrary no increase in local or systemic adverse events was reported in children who received 2 doses of 0.5 ml [27]. The effectiveness of influenza vaccines also depends on the match between the vaccine and the circulating strains. The effectiveness of the virosomal adjuvanted vaccine was studied in almost 2000 Spanish children (of 3-14 years of age), 966 subjects received the vaccine and 985 were included in the non-vaccinated control group. The study was carried out during the season 2004-2005,

when the match was not good, nevertheless the vaccine showed an effectiveness of 75.1% in preventing cases of influenza-like illnesses and of 88.4% in preventing laboratory-confirmed cases of influenza [28].

Several studies demonstrated the immunogenicity and safety of the virosomal adjuvanted vaccine in immunocompromised children [29] and adults [30] as well as in subjects with chronic diseases, as diabetes [31], asthma [32], cystic fibrosis [33], and decompensate cirrhosis [34].

The humoral and the cellular immune response of the virosomal adjuvanted influenza vaccine were investigated after one month in patients affected by scleroderma. Protective antibody titres for the three strains were reached in 80% of the subjects and the cellular immune response (proliferation of specific T CD4⁺ and CD8⁺ lymphocytes and production of Th1 cytokines) was found in all vaccinated subjects. During the study no progression of the course of the underlying disease was observed [35].

IMMUNOGENICITY AND EFFECTIVENESS OF VIROSOMAL ADJUVANTED VACCINES IN THE ELDERLY POPULATION

In many studies, the virosomal adjuvanted vaccines have better immunogenicity profiles with respect to non-adjuvanted conventional vaccines (whole virus and subunit) [36-38].

In a study by Gluck et al. the virosomal adjuvanted influenza vaccine was compared with an inactivated whole vaccine and a subunit vaccine and showed to induce significantly higher seroconversion rates (83%, 79% and 67% for A/H1N1, A/H2N2 and B respectively) than the comparators for all three strains. Also increase in GMT was significantly greater with the virosomal adjuvanted influenza vaccine. At baseline more than 50% of subjects had protective antibody titres before immunisation for the A/H3N2 strain and less than 10% of subjects had protective antibody titres for the A/H1N1 and B strains, seroprotection rate after vaccination was 72% and 54% for the A/H1N1 and B strains respectively. This rate was significantly superior for the virosomal adjuvanted vaccine with respect to the subunit vaccine [36].

In a following study other authors compared the virosomal adjuvanted influenza with a subunit vaccine. The seroconversion rates for both A/H1N1 and A/H3N2 strains were significantly higher for the virosomal adjuvanted vaccine (71 and 94.7% respectively versus 32.3 and 61.7% in the subunit group, $p < 0.005$) [37].

In 2005 deBruijn et al. reported that 4 months after the immunization with the virosomal adjuvanted vaccine the immune response is still at protective levels [38].

The better tolerability profile of the virosomal adjuvanted vaccine with respect to the other adjuvanted influenza vaccine on the market (MF59 adjuvanted influenza vaccine) is demonstrated in many studies [39, 40].

In a double blind clinical study performed during the influenza season 2002-2003, comparable seroprotection rates for 3 strains (A/H1N1, A/H3N2 and B) were shown after elderly patients (mean age 76) when immunized with Inflexal V[®] (virosomal adjuvanted vaccine) and Flud[®] (MF-59 subunit adjuvant vaccine) influenza vaccines, but the first vaccine caused pain at injection

site significantly less frequently than the comparator. Also the number of days with medications to treat a vaccine related adverse event was significantly lower with the virosomal adjuvanted vaccine [39].

In another comparison study, conducted during the same season, a conventional split vaccine, a virosomal adjuvanted and a MF59 adjuvanted vaccine showed to be highly immunogenic, safe and well tolerated. A higher rate of solicited local symptoms was reported by the group who received the MF59 adjuvanted vaccine [40]. Pegliasco et al. demonstrated that the adjuvanted vaccines (MF59 and virosomal) induced a higher antibody response than whole virus vaccine in institutionalised elderly and that there was no significant difference between groups that received MF59 and virosomal adjuvanted vaccines both 4 and 12 weeks after the immunization [41].

The efficacy of the virosomal adjuvanted vaccine in preventing Influenza Like Illness (ILI) and the safety of the concomitant administration of the virosomal adjuvanted vaccine and anti-pneumococcal vaccine were investigated in non-institutionalised subjects. The incidence of ILI was significantly higher in the non-vaccinated control group (17.3%) than in two vaccinated group (6.6 and 4.3% in the elderly vaccinated with influenza vaccine alone and with influenza plus pneumococcal vaccine respectively). The side effects were mild and did not require medical assistance [42].

In another study the virosomal adjuvanted showed a protective efficacy of 80.6% in reducing the incidence of ILI in elderly [43].

A cost/benefit analysis compared the virosomal adjuvanted vaccine with the conventional vaccines (split and subunit vaccines). The simulation for the influenza vaccination campaign in elderly show that the saving for the national health system would be of 124 euros and 108 euros per vaccinated subject using the adjuvanted and conventional vaccines respectively [44].

Conclusions

The inactivated vaccines on the market offer a great deal of protection against influenza by limiting disease severity and reducing the potential for severe disease complications but these vaccines are not optimal for older adults due to their waning immunity and other factors affecting immunogenicity such as comorbid conditions. There is a clear need for vaccines which are able to evoke the immune response to influenza in the elderly population and in those at high risk for severe complications.

Currently available data suggest improved immunogenicity of the virosomal adjuvanted vaccine in the elderly and in subjects without protective antibody as compared to conventional vaccines. The tolerability profile of the virosomal adjuvanted vaccine is comparable with the conventional vaccines and better than the MF59 adjuvanted vaccine.

Finally, use of virosomal adjuvanted vaccines in vaccination campaigns targeting the elderly population would translate into greater savings for the National Health System with respect to conventional vaccines.

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