

REVIEW

Utility of virosomal adjuvated influenza vaccines: a review of the literature

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Key words

Influenza • Influenza vaccine • Virosomal adjuvated vaccine

Summary

In spite of the efforts of the World Health Organization (WHO), influenza continues to be a major public health problem, both because of its impact on the health of subjects at risk, such as the elderly, and because of the economic burden that it places on society.

Adjuvants are agents which, when incorporated into vaccines, enhance the immunogenicity of their antigens. The need for ever more immunogenic and efficacious influenza vaccines has led to

the development of innovative vaccines. One of these, the virosomal vaccine, has been on the market since 1997.

The results obtained through controlled clinical studies and widespread application in the field suggest that the virosomal vaccine is not only an important tool for the prevention of seasonal influenza but also a valid means of potentiating the effect of a pandemic influenza vaccine and, perhaps, of preparing multivalent or combined vaccines.

Introduction

In spite of the efforts of the World Health Organization (WHO), the political commitment of its member states, scientific progress and the impressive technological advances of the vaccine industry, influenza continues to be a major public health problem, both because of its impact on the health of subjects at risk, such as the elderly, and because of the economic burden that it places on society.

In the USA, influenza and its complications are responsible for about 186,000 additional hospitalizations and 44,000 deaths among the elderly (> 64 years) every year [1]. The risk of dying from influenza in the United States is 16 times higher among subjects over 84 years of age than among those aged from 65 to 69 years [2].

Among children under the age of 5 years, influenza is a frequent cause of medical examination and hospitalization. In the USA, the incidence of emergency room admissions for influenza among children is estimated to have ranged from 6 to 27 per 1000 in the 2002-03 and 2003-04 winter seasons [2]. The rate of hospitalization due to influenza is substantially similar to that of subjects at risk, including those over 64 years of age [2]. As is well known, the following categories are at risk: pregnant women, subjects with pulmonary, cardiovascular, kidney, liver, hematological and metabolic diseases, immunodepressed patients, ecc.

Influenza vaccines are safe and reasonably efficacious; indeed, in most advanced countries, elderly people are regarded as a risk group for whom vaccination is a priority. Nevertheless, from 2005 to 2007, the efficacy of vaccinating such subjects was hotly debated, especially

after a study by Jefferson et al. [3] that had been published in the *Lancet* in 2005.

In order to make a substantial contribution to the issue, Nichol et al. conducted a survey of 713,872 elderly members of the general population in the United States from 1990 to 2000. They found that, during those 10 seasons, influenza vaccination was associated with a significant reduction (27%) in the risk of hospitalization and of death (48%) [4]. Previously (1995), Gross et al. [5] had carried out a meta-analysis of cohort studies, which had revealed a 50% reduction in hospitalizations and a 68% reduction in mortality. In other studies, conducted in Liguria, vaccine efficacy in the elderly was estimated to be 57.11% (1999-2000 season) and 45.74% (2000-2001 season) [6-7].

However, it is well known that vaccines must be continually updated, and that when the "forecasted" viruses do not closely correspond to those actually in circulation vaccination proves to be suboptimal; consequently, there is plenty of room for improvement.

Moreover, considering the risk of periodic pandemics, in recent years adjuvated vaccines have been developed and authorized for marketing in Europe. One of these is the virosomal adjuvated vaccine, which is now authorized for administration to all age-groups and is particularly indicated for subjects at risk.

The present critical review of the scientific literature examines the characteristics of the virosomal adjuvated vaccine, the results yielded by controlled clinical studies and the pharmaceutical-economic implications, and makes an appraisal of the use of this product since 1997 and of its future prospects.

Adjuvants

Adjuvants are agents which, when incorporated into vaccines, enhance the immunogenicity of their antigens. The first insights into this phenomenon are attributable to Gustave Ramon in 1925 [8]; however, it was Glenn who, the following year, discovered the adjuvating effect of aluminum salts [9]. The most recent vaccines are highly purified; this has almost totally eliminated the exotoxins, endotoxins, extraneous proteins, etc, which exerted an intrinsic adjuvating action on vaccines [10]. With regard to influenza, the first preparations, which were chemically purified, contained many egg proteins. Advances in the purification of this vaccine have made it possible to achieve greater tolerability. Nevertheless, the need for ever more immunogenic and efficacious influenza vaccines, especially for subjects at risk, has prompted the development of alternative vaccines; these are adjuvated not only with aluminum salts, but also with new preparations such as squalene and phospholipids (virosomes) [11, 12].

Liposomes and virosomes

In 1974 Allison and Gregoriadis discovered the adjuvating role of liposomes [12]. In 1992 the first vaccine adjuvated with virosomes was proposed. This was a hepatitis A vaccine [13]. The mechanism of action was not well known at the time and was attributed to a depot effect involving the slow release of the antigen, and to the ability of the antigen and the vesicles to migrate to the regional lymph-nodes after injection.

Subsequently, virosomes were used in the preparation of influenza vaccines. Technically, the influenza virosomes are spheres of lipid vesicles with a mean diameter of 150 nm (Figs. 1, 2), from whose surface emerge spicules of 10-15 nm. They are prepared by removing the surface glycoproteins of the influenza virus by means of detergents; these glycoproteins are then mixed with natural and synthetic phospholipids such as phosphatidylcholine and phosphatidylethanolamine [13].

Thus, the virosome presents as a vesicle delimited by a lipid monolayer which is very similar to that of the cell membrane. Structurally and functionally, the virosome is a reconstituted sheath of the influenza virus, bereft of genetic material.

Phosphatidylethanolamine is able to directly stimulate the B cells to produce antibodies against the antigens present on the virosome without inducing an antibody response against the phospholipids that make it up. The presence of the surface antigens of the influenza virus on the virosome then stimulates the phagocytosis of the presenting antigen cells, such as macrophages and dendritic cells.

Wilscht and McElhaney [14] have recently hypothesized that, as a result of the repetitive arrangement of the hemagglutinin on their surface, virosomes interact very efficiently with the immunoglobulin receptors of the B lymphocytes. Moreover, virosomes are thought to be avidly captured by the cells presenting the antigen, in particular the dendritic cells. The antigens present on the surface of the virosome, and those derived from its degradation, penetrate the MHC II (Major Histocompatibility Complex II) class cells and activate the T-helper cells. In addition, through the fusion of the virosomes, the antigens inside the virosome enter into the cytosol, activating the MHC I (Major Histocompatibility Complex I) cells of innate immunity and the cytotoxic T lymphocytes. Thus, virosomes display the characteristics of an adjuvating system, are biodegradable, atoxic and do not induce the formation of antibodies against themselves [15].

Finally, virosomes appear to be a flexible platform that is particularly suited to the potentiation of prophylactic, therapeutic and combined synthetic vaccines [16]. Indeed:

- their virus-like structure provides the B lymphocytes with a repetitive antigenic presentation and mimics the natural presentation of the antigen, which makes for a humoral response that is specific and of high quality [17, 18];
- the functional activity of fusion of the virosomes activates the receptors, giving rise to a natural process of intracellular elaboration of the antigen and to activation of both humoral and cellular immunity;

Fig. 1. Architectural structure of the virosomal adjuvated influenza vaccine on electron microscopy (Electron Microscopy Unit of the Department of Health Sciences - DISSAL – University of Genoa).

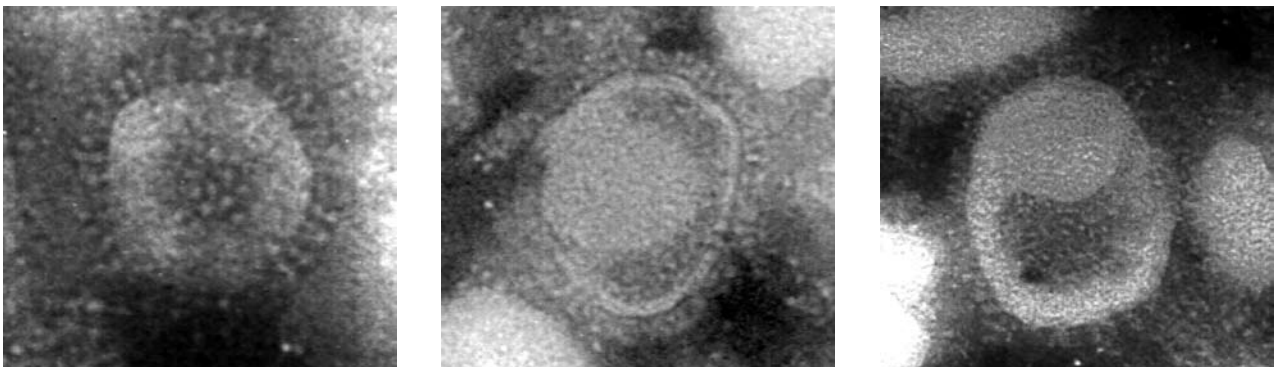
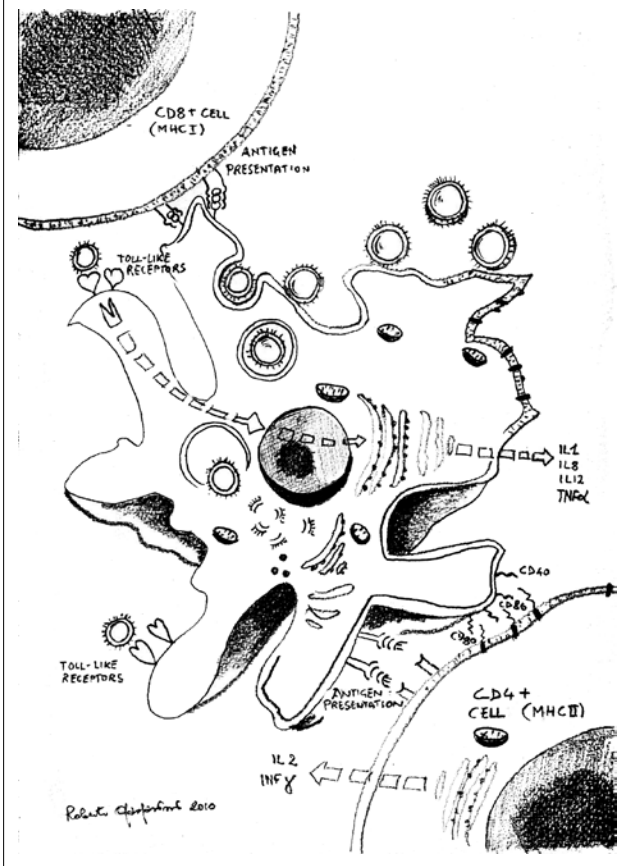


Fig. 2. Mechanism of stimulation of a dendritic cell by virosomes. The virosome enters the cell and forms an endosome. From the endosome, the virosome is released into the cytosol; this is the site of the antigenic rearrangement that leads to presentation to the MHC II cells (CD4+), which, following stimulation, produce lymphokines such as interleukin 2 and gamma interferon. The differently elaborated antigen is presented to the MHC I cells, stimulating innate immunity. The virosome binds to a toll-like receptor and this induces the production of pro-inflammatory lymphokines such as interleukin 1, 8, 12 and TNF-alpha.



- the antigen is partially protected from extracellular degradation; this allows more prolonged stimulation of the immune system;
- virosomal vaccines seem to be particularly useful in children, the elderly (immuno-senescent) and immunodepressed subjects.

To date, virosomal vaccines have been developed for hepatitis A and influenza; millions of doses have been administered and their safety and tolerability profile has proved to be very good.

Virosomal influenza vaccines: past, present and future

The first important experimental study of a virosome-adjuvated vaccine in humans was conducted by Gluk et al. in 1994 [19]. The study compared a virosomal vaccine with an inactivated whole vaccine and a subunit vaccine, and showed that the virosomal vaccine was able

to induce significantly higher antibody titers, seroconversion and antibody responses to all three components contained in the vaccine (H1N1/Singapore 6/86, H3N2/Beijing 353/89 and B/Yamagata 16/88) among the residents of a facility for elderly people.

Later, Conne et al. [20] conducted a double-blind study on geriatric patients. They found that, although both vaccines studied (virosomal and subunit, containing the strains A/Singapore 6/86, A/Beijing 32/92 and B/Panama 45/90) elicited significant increases in the geometric mean titer for the three viral strains, the virosomal vaccine induced an antibody response (4-fold increase in the titer) for the A viruses in a significantly higher number of recipients. With regard to the B virus, the percentage of subjects displaying protective titers ($\geq 1/40$) one month after vaccination was significantly higher among subjects who had received the virosomal vaccine. Finally, of those subjects who had had low antibody levels before vaccination ($< 1/40$), a statistically higher number displayed protective levels for both A viruses when they had received the adjuvated vaccine. The authors underlined the fact that 68.4% of the subjects vaccinated with the virosomal vaccine displayed protective antibody levels towards the three viruses, as against only 38% of those vaccinated with the subunit vaccine.

In a study published in 2001, Pregliasco et al. [21] compared a virosomal vaccine with a whole virus vaccine and a vaccine adjuvated with squalene (MF59). The results showed that both the virosomal vaccine and the squalene-adjuvated vaccine elicited greater antibody responses than the inactivated whole vaccine.

Mensi et al. [22] studied the cell-mediated response in two groups of elderly subjects after vaccination with a virosomal vaccine and a whole vaccine. Innate immunity, which is of fundamental importance in defense against viral diseases, was analyzed by assaying interleukin 2 (IL-2) and gamma interferon (IFN γ), while adaptive immunity (which leads to the production of antibodies, but which needs to be triggered by innate immunity) was analyzed by assaying interleukin 4. The results of the study showed that the virosomal vaccine was able to activate both innate and adaptive immunity, the logical consequence of which was an increase in specific antibody titers. The whole vaccine proved to be less efficacious in inducing the production of antibodies.

In the 2000/2001 season, experimentation involving 363 subjects revealed an 80.6% level of protective efficacy of a virosomal vaccine in terms of a reduced incidence of flu-like illness [23].

Another comparative study of a virosomal vaccine and a vaccine adjuvated with MF59 (FLUAD) [24] showed that both vaccines stimulated the production of antibodies to levels above the minimum thresholds of protection established by the *ad hoc* EMEA commission [25] for the three antigens contained in the vaccine and recommended by the WHO for the 2002/2003 season. Moreover, it was seen that at least 90% of the subjects involved proved to be sero-protected 28 days after vaccination. The rates of sero-protection were comparable between the two vaccines and both vaccines displayed a very

good safety profile. However, the virosomal vaccine was better tolerated: pain at the injection site, systemic reactions and the administration of drugs to treat adverse reactions were all significantly lower in the virosomal vaccine group.

In the 2002-03 season, Consonni et al. [26] studied 347 elderly subjects who were vaccinated with either a virosomal vaccine alone (166 subjects) or the virosomal vaccine and a 23-valent pneumococcal vaccine (139 subjects). A group of 69 unvaccinated subjects served as controls. All subjects were followed up with respect to clinical symptoms over the winter season. Flu-like symptoms were recorded in 6.6% of the subjects who had received the virosomal vaccine alone; among those who had received both vaccines, the incidence was 4.3%, while in the control subjects it was 17.3% (the difference from the two previous groups being highly significant). This study also confirmed the very good safety and tolerability profile of the virosomal vaccine.

In an open controlled clinical study conducted in the 2002/2003 season on 840 elderly subjects, a split vaccine, a vaccine adjuvated with MF-59 and a virosomal vaccine were administered to three groups of volunteers [27]. The three preparations displayed high immunogenicity and an acceptable tolerability. Local side-effects were more frequent when the MF-59-adjuvated vaccine was administered.

In 2005 de Bruijn et al. [28] evaluated three clinical trials involving virosomal vaccines. In one of the trials, 926 adults (18-60 years) received either the virosomal preparation or a conventional subunit vaccine. In another trial, a virosomal vaccine and a split vaccine were compared in participants (87) over 60 years of age. The third trial aimed to check immunogenicity after one year in adults (42 subjects, 18-60 years) revaccinated with a virosomal vaccine. Having analyzed these studies, the authors concluded that:

- the virosomal vaccine was safe and efficacious in all subjects studied, including those at risk of influenza-related complications;
- the virosomal vaccine was better tolerated than the subunit vaccine at the local level;
- for the A-H3N2 strain, the virosomal vaccine stimulated the production of antibodies, the levels of which remained high over time, regardless of the age and state of health of the recipients.

Other studies have documented the good tolerability and immunogenicity of virosomal vaccines in children, adults and subjects at risk. This is important in that influenza in children can have particularly serious consequences [29-34]; moreover, it has been hypothesized that in some categories of subjects at risk, such as asthmatics, the vaccination might facilitate the acute recurrence of the allergic disease. In 2004 Kanra et al. [35] published a study carried out on 453 children aged between 6 and 71 months, in which very good immunogenicity was observed. More recently (2010), Esposito et al. [36] studied the immune response to and tolerability of a double dose of virosomal vaccine (0.5 ml) in children aged less than one year. They found that there was no significant increase in side-effects in comparison with the standard

dose (0.25 ml), while both the short- and long-term antibody response increased significantly.

Salleras et al. [37] used both clinical and laboratory methods (Real Time Polymerase Chain Reaction, RT-PCR) to evaluate the clinical efficacy of a virosomal vaccine in children aged 3-14 years in September-October 2004. The study revealed a vaccine efficacy of 75% in preventing cases of influenza-like illness (ILI) and of 88% in preventing laboratory-confirmed cases.

In a study published in 2000 [38] involving children and adolescents with cystic fibrosis, administration of a virosomal vaccine proved to be safe and to have a strong immunogenic effect. In children (3-9 years old) with asthma, vaccinated in the 2005-2006 winter season [39], very good tolerability and immunogenicity were recorded. Nevertheless, in children without antibodies before vaccination, protection declined markedly after six months; this underscores the need for a booster dose one month after the first dose, and the advisability of subsequent annual revaccination.

In asthmatic children, Esposito et al. [40] confirmed the very good tolerability of the vaccine both in children with asthma and allergy to eggs (mean age 6.03 years) and in those with persistent asthma but without allergy to eggs (mean age 6.34 years). In addition, the vaccine has proved to be safe, well tolerated and endowed with good immunogenic power both in children [41-43] and in HIV-positive adults [44].

The vaccine is therefore well tolerated by subjects with conditions that are at risk, including those with scleroderma, as demonstrated by Setti et al. [45] in subjects over the age of 18 years who had suffered from the disease for at least six months. Finally, Gaeta et al. [46] also demonstrated the immunogenicity and safety of the vaccine in subjects with decompensated cirrhosis of the liver.

Economic studies

The economic benefits attributable to virosomal influenza vaccines have mainly been studied with regard to children [47, 48]. However, a study conducted by Gasparini et al. [49] involved elderly non-institutionalized subjects.

In the above-mentioned study conducted by Salleras et al. [37], comparison between vaccinated and unvaccinated children revealed the advantages of vaccination: a 20% reduction in the consumption of antibiotics, a 46.9% reduction in absences from school, and 28% less absenteeism from work on the part of other family members. In similar studies carried out by Principi et al. [50, 51] on children between 2 and 5 years of age who had received two doses of virosomal vaccine, the consumption of antibiotics fell by 32%, school absences by 48% and maternal absenteeism from work by 33%.

Marchetti [52] et al. used a Markovian simulation model to assess the cost-effectiveness of a virosomal vaccine. They found that the vaccine was cost-saving from the point of view of society and cost-effective (from € 10,000 to € 13,333 per year of life gained in good health

[QUALY]) from the standpoint of the third-party payer (National Health Service [NHS]).

The study conducted on elderly subjects by Gasparini et al. [49] was a cost/benefit analysis from the standpoint of the NHS as the third-party payer. The virosomal vaccine was compared with the conventional vaccine (subunit or split). The simulation of the study enabled the authors to conclude that the virosomal vaccine yielded a saving of € 124 (purchasing power as of 2002) versus € 108 yielded by the conventional vaccine. Moreover, sensitivity analysis revealed that, even when the efficacy of the virosomal vaccine was considered to be 46%, it was still more advantageous than that subunit or split vaccine.

Conclusions and prospects

The experiences quoted indicate that virosomal vaccines hold out good hopes of overcoming some critical aspects of vaccination for the elderly, one of which is immunosenescence. This type of vaccine is very well tolerated owing to the characteristics of the virosomes, which are very similar to the composition of the cell membrane.

The mechanism of action of a good adjuvant must always be suitably balanced. Indeed, in anti-viral vaccines the adjuvant must first of all stimulate innate immunity and then the MHC I class cells, which are essential in killing infected cells and coordinating their elimination. These MHC I class cells produce pro-inflammatory lymphokines and chemokines, which recruit other cells that play a role in the immune response [53]. These lymphokines and cells are also responsible for the side-

effects of vaccination, which are usually evident at the local level, such as pain, redness and swelling. However, the adjuvant must also activate the regulatory cells in an appropriate manner, so that they can correctly temper the immune response, thereby limiting the time and intensity of the immune reaction. Moreover, activation of the MHC I cells conditions the adaptive response and the consequent production of antibodies in such a way as to ensure a consolidated, long-standing (e.g. 12 months) response.

Virosomal influenza vaccines have been on the European market for more than 10 years, and more than 41 million doses have been sold. Post-marketing surveillance has shown that they have an excellent safety and tolerability profile; this is also due to their purity, the absence of formaldehyde and thiomerosal, their very low content of egg protein and their biocompatibility.

The production of antibodies, which, in the final analysis, expresses the activation of adaptive immunity, is better elicited by virosomal vaccines than by whole or subunit or split vaccines, particularly with regard to the viruses A/H3N2 and B. Indeed, conventional vaccines have often proved poorly immunogenic against this latter type of virus.

In conclusion, within the wide range of influenza vaccines available, virosomal vaccines display very good tolerability and high immunogenicity in subjects of all age-groups as well as in subjects with pathologies that constitute risk factors for post-influenza complications, such as asthmatics, those with scleroderma and HIV-positive subjects. This means that virosomal vaccines have great flexibility and are particularly useful in seasonal immunization programs.

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