

Inflexal[®] V—The influenza vaccine with the lowest ovalbumin content

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Abstract

The antigen used in influenza vaccines is directly derived from influenza viruses. It is produced in embryonated chicken eggs for both inactivated and live attenuated influenza vaccines. As a consequence, all influenza vaccines contain varying amounts of residual egg proteins, depending on the specific manufacturing process of the vaccine. The degree of purity of influenza vaccines can be assessed by quantifying the total amount of protein in relation to the amount of specific antigen. Alternatively, ovalbumin can be used as surrogate marker, representing the amount of egg-derived proteins present in the vaccine. Egg proteins, such as ovalbumin, are classified as sensitising agents. Their presence in a vaccine may be linked to adverse reactions. Such a vaccine is not suitable for subjects with a known history of egg-allergy. This population is currently excluded from influenza vaccination programs. Influenza vaccines are intended for annual re-immunisation. This repeated administration may lead to an immunity against ovalbumin and other egg proteins, which in turn may provoke increased local and systemic reactions and a reduced tolerability profile of the product.

Comparing several influenza vaccines present on the market, ELISA and Western blot analysis showed clearly a very low level of ovalbumin in Inflexal[®] V. Furthermore, data showed, that Inflexal[®] V is the influenza vaccine with the lowest ovalbumin content.

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1. Introduction

Egg allergies occur in approximately 0.5% of the population and in approximately 5% of atopic children [1]. Residual quantities of egg proteins found in the influenza vaccine are sufficient to induce severe and rarely fatal hypersensitivity reactions in children with egg allergies [2]. The purity of Inflexal[®] V is reflected in its low ovalbumin content, an indication of the amount of egg protein and other impurities in the vaccine. Inflexal[®] V contains less than 10 ng of ovalbumin per dose, satisfying the European Pharmacopoeia requirements (<50 ng per dose, season 2004/2005) for virosomal influenza vaccines. In contrast, conventional influenza vaccines are permitted to contain up to 1000 ng ovalbumin per dose. It is expected that the virosome-based influenza vaccine with this low ovalbumin content would induce less allergic reactions.

The American Academy of Paediatrics recommends that patients with severe, anaphylactic reactions after egg ingestion should not receive an influenza vaccine until they have been appropriately skin tested with a diluted preparation of vaccine. If there is a positive skin test response to the vaccine preparation, either by the epicutaneous or intradermal skin testing method, the influenza vaccine should not be administered.

1.1. Inflexal[®] V production process

Following the production techniques used for individual monovalent concentrates of the virosomes, each of the three monovalent influenza virus strain bulks, currently H1N1, H3N2 and B, are used as the starting material for the manufacture of Inflexal[®] V. The manufacturing process for Inflexal[®] V as described by Mischler and Metcalfe [3] is comprised of two production blocks: purified and inactivated influenza whole-virus antigen, and the formulation of the influenza gly-

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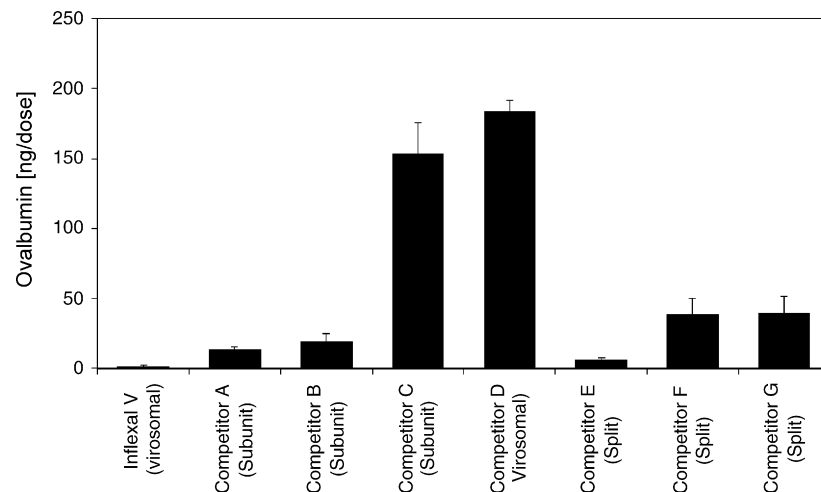


Fig. 1. Ovalbumin content analysed by a Sandwich-ELISA from different influenza vaccines (season 04/05). From each competitor one lot was 3-times independently analysed. The type of vaccine is indicated between brackets.

coprotein subunits. The lot consistency and purity of each of the virosomal products is represented in published data [3]. It can be seen that, throughout several consecutive lots for each of the 3 influenza strains chosen, there is very little variation in the ratio between HA and phospholipids which form the virosomes. In addition, the virosome pools are also free from detergents (Octaethylglycol-mono-(*N*-dodecyl)-ether: OEG). The high purity of the influenza surface glycoproteins present in Inflexal® V has been ascertained by polyacryl gel electrophoresis and total protein determination. The results of the gel electrophoresis have fulfilled all the requirements outlined by the WHO and European Pharmacopoeia.

A comparison of the ovalbumin content in several influenza vaccines present on the market is shown in the following chapters.

2. Material and methods

Eight commercial available influenza vaccines samples of season 2004/2005 (one lot each) have been analysed by an ELISA, SDS-PAGE and Western blot method for their ovalbumin content. Samples were Inflexal® V (Berna Biotech), Fludac® (Chiron), Agrippal® (Chiron), Influvac® (Solvay), Invivac® (Solvay), Fluarix® (GSK), Mutagrip® (Sanofi-Pasteur) and Begrivac® (Chiron).

2.1. ELISA

In order to determine the amount of ovalbumin, a Sandwich-ELISA was applied. A microtiter plate was coated with a polyclonal antibody (guinea-pig) against ovalbumin. The polyclonal antibody can therefore capture the ovalbumin present in the samples. A monoclonal antibody against ovalbumin (rabbit) is added and binds to the captured ovalbumin. The monoclonal antibody is coupled to an anti-

rabbit-IgG-HRPO and leads to an indirect measurement of the ovalbumin amounts present in the samples. The samples are diluted in order to be in the linear range of the assay. This method is validated according to the ICH guidelines for Inflexal® V matrix, but not for the other matrices of the other samples.

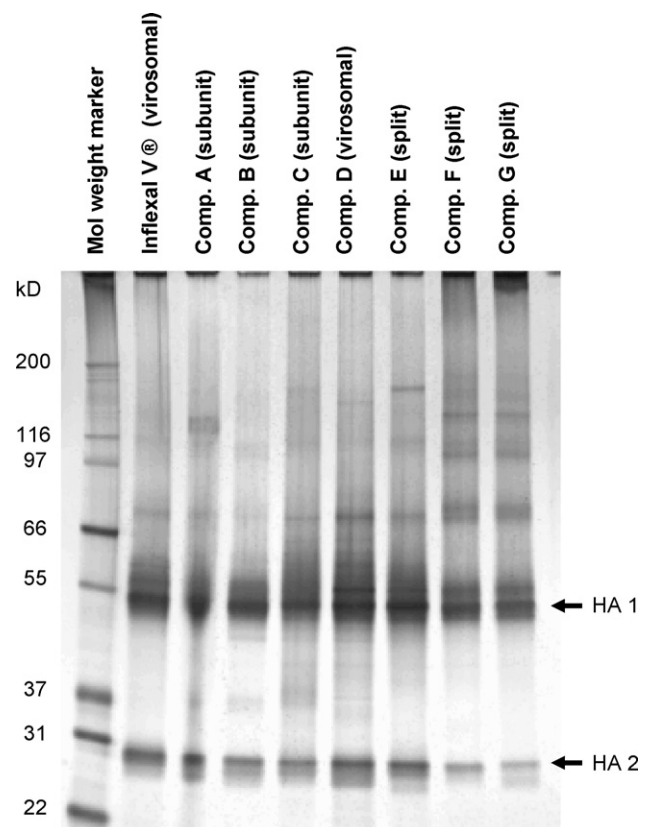


Fig. 2. SDS-PAGE stained with Silver stain reagents (reducing conditions, 4–12% gradient gel). The same vaccine lots were loaded as used for ELISA analysis (Fig. 1).

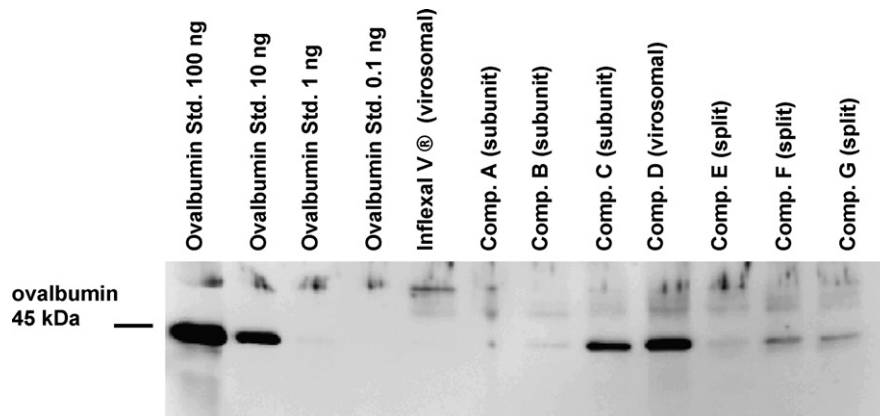


Fig. 3. Western blot anti-ovalbumin analysis. Reducing SDS-PAGE (12%) was transferred to a Western blot membrane. Detection was performed with a polyclonal anti-rabbit serum. As a second antibody, an anti-rabbit HRPO conjugate was used and detection was performed by chemiluminescence. The same vaccine lots were loaded as used for ELISA (Fig. 1) and SDS-PAGE (Fig. 2) analysis.

2.2. SDS-PAGE

Samples (10 μ L) were loaded on a 4–12% gradient SDS-Gel under reducing conditions. After the run, the gel was stained with silver stain according to the manufacture protocol.

2.3. Western blot

Samples (10 μ L) and ovalbumin standard (Sigma) were loaded on a 12% gradient SDS-Gel under reducing conditions. After the run, the gel was transferred to a Western blot membrane. A polyclonal anti-ovalbumin antibody (rabbit) was added, detection was done with an anti-rabbit HRPO-conjugate. Visualisation was performed with a chemiluminescent substrate and exposure of chemiluminescence was measured by an imager system.

3. Results

3.1. Characterisation of ovalbumin content by ELISA in eight influenza vaccines

Screening eight commercial available influenza vaccines of the season 2004/2005 by ELISA revealed a wide variety of ovalbumin contents (Fig. 1). Two subunit vaccines (both from the same manufacturer; one a pure subunit formulation, the other a virosomal formulation) showed comparable high ovalbumin contents (subunit: 153 ng ovalbumin/0.5 mL; virosomal: 184 ng ovalbumin/0.5 mL). Substantial less ovalbumin was detected in all other vaccines, varying from 6 ng/0.5 mL (competitor A) to 40 ng/0.5 mL (competitor G). The influenza vaccine with the lowest ovalbumin content detected by ELISA was clearly Inflexal[®] V (1 ng ovalbumin/0.5 mL). No tendencies of the ovalbumin content could be observed dependent of the production process (subunit or split).

3.2. Characterisation of total protein by SDS-PAGE in eight influenza vaccines

Different manufacturing processes are used for the production of influenza vaccines. Such differences can be made visible also by different protein patterns when samples are loaded onto a reducing SDS-PAGE and stained afterwards with silver-stain (Fig. 2). The proteins haemagglutinin and neuraminidase are most abundant proteins, but several other proteins can be distinguished. Protein patterns do greatly vary.

3.3. Characterisation of ovalbumin content by Western blot in eight influenza vaccines

As an alternative method for ovalbumin detection, Western blotting using specific anti-ovalbumin antibodies was performed with the same eight samples as used by the ELISA technique (Fig. 3). The results are very similar, although quantification is hard to do. Also by Western blot, vaccines from competitor C and D (lane 8, lane 9) do have the highest amount of ovalbumin; there is no ovalbumin band detectable for Inflexal[®] V (lane 5). The intensity of the chemiluminescence by Western blot and the ELISA results do in fact show the same pattern, therefore one can conclude this measurements as meaningful.

4. Conclusions/discussion

The tolerability of prophylactic vaccines is of increasing importance; particularly sensitising agents should only be present in low amounts. The European Pharmacopoeia (EP) requirement for split and subunit influenza vaccines does allow up to 1 μ g ovalbumin per dose [4,5]. None of the analysed competitor vaccines does contain such high amounts. The highest amount of ovalbumin for split and subunit vaccines was found in the influenza vaccine from competitor C

with 153 ng ovalbumin/dose (0.5 mL). All the other vaccines have less than 50 ng ovalbumin/dose.

4.1. *Inflexal[®] V has the lowest ovalbumin content; other virosomal vaccines have until 200-times more*

For virosomal vaccines, the EP requirement allowed until the end of 2005 not more than 50 ng ovalbumin per dose [6]. Inflexal[®] V does meet this specification by far: with 1 ng/dose Inflexal[®] V is decidedly the influenza vaccine with the lowest ovalbumin content and about 200-times lower than other influenza vaccines present on the market. Surprisingly, the virosomal vaccine from competitor D shows the highest ovalbumin content from all vaccines analysed: 184 ng ovalbumin/dose. This is nearly 4-times higher than the EP requirement allowed for season 2004/2005, and dramatically higher than the ovalbumin content in Inflexal[®] V. However, the European Pharmacopoeia Version 5.3 (from 01.01.2006) does allow up to 1 µg ovalbumin per human dose also for virosomal vaccines.

4.2. *Why does Inflexal[®] V have such a low ovalbumin content?*

For the formation of virosomes, the influenza virus, grown in hen's egg, is first inactivated with beta-propiolactone. The influenza surface antigens Neuraminidase and Haemagglutinin are then purified by the production steps developed by Berna Biotech Ltd. and mixed with phospholipid components [3]. This procedure results in a high purity grade for Inflexal[®] V. Monovalent pools do contain high amounts of ovalbumin (150–900 ng ovalbumin/mL) which are then continuously reduced to less than 10 ng ovalbumin/dose (0.5 mL)

in the final formulated bulk. Several ultracentrifugation steps provide a high degree of purity. The analyses of the data presented here are consistent with the earlier published results from Mischler and Metcalfe [3]. Ovalbumin can be seen as an important surrogate marker for general impurities in a vaccine deriving from hen's eggs. By demonstrating low ovalbumin presence, one might conclude also a general low level of other impurities.

In summary, Inflexal[®] V is the influenza vaccine with the lowest ovalbumin content, competitor vaccines do contain 6- to 200-times more ovalbumin and Inflexal[®] V has been proven to be a safe vaccine (total 19 Mio. doses sold) with a spontaneous ADR reporting rate of 0.9 cases/100,000 doses sold. Data presented here and in an earlier publication [3] describe a consistent and stable procedure for the production of Inflexal[®] V.

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