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Quality assessment of DNA vaccines: hepatitis-B vaccine*

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Abstract: Vaccines manufactured following "classical" methods contain inactivated or infectious but attenuated viruses or bacteria. In some instances, the inactivated agents are purified. In other cases, the vaccines contain protein subunits or practically pure polysaccharides. It is generally accepted that the final product cannot be completely characterised and that therefore extensive "in-process" controls are necessary to prove the consistent quality of such vaccines. Control tests are carried out on the substrate, the pooled bulk vaccine and on the final containers.

Vaccines produced by recombinant DNA techniques consist of pure proteins. The production is carried out by the multiplication of the "working seed" under well-defined standardised conditions followed by clarification, extraction, purification, formulation. "In-process" controls are incorporated at each step and specifications for acceptance are formulated.

The biological methods used for the classical vaccines are completed by physico-chemical and immunological determinations of antigen content, identity and purity for the "new generation" products.

The requirements for the manufacturers are based on the documents issued by the World Health Organisation and by the national control authorities. The marketing of vaccines is based on a lot by lot release procedure, whereby each lot is tested by the manufacturer and the national control authority before use.

Hepatitis-B vaccine, derived from transformed yeast cells, is the first and sole vaccine which has obtained a world-wide license. The quality assessment of this vaccine has been achieved following the requirements for the new generation of biomolecules. It is an example for future vaccines.

Keywords: *Quality; vaccines; recombinant DNA; hepatitis-B.*

Introduction

Vaccines have been used for about 200 years. The development has always been linked to available techniques for obtaining the adequate antigen or immunogen. In bacterial vaccines, the evolution of technology has provided the world with vaccines containing whole cell microorganisms, detoxified toxins, purified, chemically defined, cell wall polysaccharides and isolated, purified protein antigens. For viral vaccines, the development has been directed by the availability of isolation and multiplication technology and the presently available vaccines are produced in a variety of substrates:

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living animals, embryoned eggs, cell cultures. Recombinant DNA technology has produced one licensed vaccine, namely hepatitis-B, extracted from transformed yeast cells. The vaccines obtained by the different technologies in use differ in the requirements for their quality assessment. This paper considers in detail the principal factors involved in the quality assessment of recombinant DNA with reference to a hepatitis-B vaccine.

Overview of vaccines produced by "classical" methods

Vaccines manufactured following "classical" methods contain inactivated or infectious but attenuated viruses or bacteria. In some instances, the inactivated agents are purified. In other cases, the vaccines contain subunits or practically pure polysaccharides. It is generally accepted that the final product cannot be completely characterised and that therefore extensive "in-process" controls are necessary to prove the consistent quality of such vaccines.

The requirements for the manufacturers are based on the documents issued by the World Health Organisation and by the national control authorities. The marketing of vaccines is based on a lot-by-lot release procedure, whereby each lot is tested by the manufacturer and the national control authority before use.

The quality of vaccines is generally determined by three criteria: safety, purity, immunogenicity. For viral vaccines where the multiplication of the active agent takes place in cell cultures of animal origin in the presence of media containing components of biological origin, such as trypsin and animal serum, the emphasis of the safety and purity testing is put on the proof of absence of extraneous agents. Extraneous agents, such as bacteria, fungi, mycoplasma and viruses, may be introduced by the cell culture itself, by the media ingredients of biological origin and by the same infectious microorganism originating from the environment. The detection of extraneous agents takes place at different production steps: the raw materials; the cell cultures before production; the bulk vaccine pool before clarification; and the vaccine in the final container.

The methods for detection use a panel of sensitive cell cultures, embryoned eggs, laboratory animals and adequate growth media. For some live vaccines, marker tests are required, as for example in the neurovirulence test for Oral Polio Vaccine. Samples of each monovalent vaccine pool are tested in comparison with an official reference vaccine by the intraspinal route in monkeys, the lesion scores induced in the neural system are recorded and then statistically evaluated.

Most viral vaccines are produced in primary cells or in validated diploid cell strains grown in chemically defined media, the resulting viral suspension is very pure after the elimination of the cell debris. The final product consists generally of a dilution of the resulting bulks. For these reasons, no additional purification steps are required.

Some inactivated viral vaccines may be produced in heteroploid cell lines or may require significant concentration of the resulting bulk suspension. For such vaccines the careful monitoring of the purification efficacy for host cell components and host cell DNA is required.

The immunogenicity of live vaccines is based on titration for the minimum infective dose of each lot. The determination of the minimum required titre is based on dose-range studies carried out during the clinical trial of the vaccine. The immunogenicity of inactivated vaccines is in most cases determined by the immune response in laboratory animals, where the new vaccine lot is tested in parallel with a validated reference

preparation. The release of the new lot is based on an accepted limit for the relative potency determined in a parallel dose–response assay. Most vaccines are sensitive to exposure at ambient temperatures, i.e. 20°C or even higher than 30°C during accidental exposure in tropical countries.

Development of stable vaccines and tests for stability are necessary to guarantee maintenance of adequate immunogenicity. This requirement is even more necessary for vaccines to be used in tropical countries and remote areas.

General quality considerations of vaccines produced by recombinant DNA technology

It is now possible to generate antigens which normally serve as components of viruses, bacteria, or protozoa by the manipulation of defined DNA coding sequences and their controlled expression in eukaryotic and prokaryotic host cells. As these antigens will constitute the active component of new vaccines, it is essential that vaccines prepared by these techniques are adequately controlled to ensure their quality, safety and efficacy.

The principles developed and successfully applied to previous generations of vaccines are invaluable for the quality control of new substances derived from recombinant DNA technology. Thus, information on the quality and purity of seed cultures, and on the effectiveness of production and testing methods are required. Furthermore, independent testing of samples taken at any point in the production process is necessary, and evidence to ascertain the consistency of the production process and the quality of the final product during full-scale manufacturing is essential. Because the production methodology is new, the control requirements and testing will, in some respects, differ from those which have been applied to vaccines made by conventional methods. Finally, the standardisation of these products will require adequate reference preparations.

Basis for specifications and standardisation

The rapid progress in the field of genetic engineering and biotechnology is illustrated by the issueing of guidelines, "points to consider" and "state-of-the-art position" documents by several national and supranational licensing and marketing authorities. The specific concerns by these groups for a new product should be considered on a case-by-case basis. Furthermore, the criteria required for a particular polypeptide depend on its intended use: dose, route of administration, duration of treatment, etc.

Compared to other biomolecules such as insulin and growth hormones, vaccines are a special class of biologicals since the dose is low (e.g. 20 μg for the recombinant hepatitis-B vaccine). For a given vaccine, the number of administrations may vary from 3 doses required to complete the basic vaccination scheme, up to 10 doses if regular booster injections are needed.

Quality assessment of yeast-derived hepatitis-B vaccines

The principle factors to be considered in assessing vaccine production can be illustrated by reference to a specific example, HBV. The production of biologicals follows the seed lot principle. Once a suitable strain has been constructed, it is essential to maintain it as the master seed lot. Starting from this master seed lot, a working seed lot must be prepared under well defined standard conditions. The working seed should consist of several hundreds or thousands of containers stored under conditions that will ensure its stability (i.e. at <-70°C).

During the fermentation cycles, the working seed will multiply in order to provide the biomass necessary for industrial production of the vaccine. The multiplication must be carefully determined so that the end product is obtained after a constant number of cycles, irrespective of the fermentation volume used.

As the manufacturing of biotechnology products comprises several steps, adequate tests and controls must be carried out at each of these production steps according to specifications based on the principles discussed above. The aim of quality assessment during production, also called "in-process" control, is to guarantee that the final products prepared in different production runs have identical quality. Detailed standard operating procedures for each of the steps ensure reproducibility, consistency, and good manufacturing practice. Furthermore, all methods must be validated during product development. Tests are carried out at each stage in order to determine effectiveness in ensuring optimal yields, elimination of contaminants, and especially the maximal recovery of the active ingredient.

Monitoring of fermentation

An important first step in biotechnical production is fermentation. In addition to the use of media with a standardised composition, the careful monitoring of temperature during the fermentation cycle and the strict application of the seed lot principle, certain tests have to be carried out during the cycle. Such controls cover monitoring of pH, dissolved oxygen, optical density, as well as the microscopical characteristics of the colonies at the seed level and at the end of the fermentation. Additional controls include sterility testing of the fermentation medium, microbiological purity of the harvest, plasmid retention of the host cell at the end of each fermentation run, and the stability of the host cell characteristics. The limits for the above parameters are determined during the development phases of the product and must be strictly adhered to in each fermentation cycle.

Purification procedures

The most important and difficult procedure is purification. This is a multistep procedure combining centrifugation, precipitation, ultrafiltration, chromatography, and other separation methods. Each step must be validated during development. Purification should eliminate the contaminants from the host cell and fermentation medium, and should remove reagents or additives used during the extraction/purification process to within acceptable limits. Yeast and vector DNA must be reduced to the picogram level.

Efficient purification will result in a purified concentrated product, which must first be assessed to fully characterise the hepatitis-B surface antigen (HBsAg) obtained; secondly, routine control tests must be carried out on each lot of purified, concentrated vaccine.

Specificity and confirmation of identity

The specificity of the expressed HBsAg is determined as follows: the stability of the seed lot is checked during storage and at the end of consecutive fermentation runs. The number of viable cells of the working seed remains between acceptable limits during storage. Fermentations carried out after different time periods give constant results meeting the specifications of the "in-process" tests and the final product analysis.

The genetic stability should be proven by nucleotide sequencing of the coding region of the constructed product and of the adjacent segments at the seed lot level, and also at

the end of a fermentation cycle. Finally, restriction endonuclease mapping of the recombinant vector at the end of representative fermentation cycles should give constant patterns.

Several useful methods for obtaining information on the chemical structure of the antigen are listed in the general guidelines already cited. The preferred methods include SDS-PAGE analysis followed by Coomassie Blue and silver staining or Western blot with polyclonal and monoclonal antibodies. The determination of the amino acid composition gives values comparable with the data obtained for the natural product or with the amino acid sequence predicted from the nucleotide sequence. Peptide mapping after cleavage with enzymes of different specificity will enable reconstruction of the predicted amino acid sequence. Finally, N/C terminal sequence determination will complete the information required on the correct expression of the antigen.

The UV spectrum of the vaccine shows the characteristic UV features of the plasma product, with maximum adsorption at 280 nm and a typical "plateau" at about 290 nm. The electron microscopic picture shows particles with a diameter of about 20 nm.

The immunological characteristics of yeast- and plasma-derived antigen can be shown by the reaction of the yeast HBsAg with poly- and monoclonal anti-plasma HBsAg antibodies. The results should show that the polyclonal antibodies perfectly recognise the yeast-derived HBsAg and that the epitopes corresponding to the plasma-derived product are also present in the yeast-derived vaccine.

Antibodies induced with the recombinant vaccine should show identity to antibodies induced by plasma vaccines and by natural infection through characterisation by: (a) determination of the percentage of antibodies raised against the common "a" determinant, considered to induce protective immunity; (b) monoclonal antibodies of defined specificity against HBV; and (c) competition studies using immunoglobulins from convalescent subjects and vaccines.

Quality control of routine production lots

In the first place, the specifications should cover the raw materials used in the different stages of the production process. The analysis of these materials is generally based on the monographs of National and Supranational Pharmacopoeiae. The control tests to be carried out on the non-adsorbed bulk and on the adsorbed vaccine in the final containers complete the routine quality control procedure.

The relevant tests to be carried out and the limits for acceptance have to be determined in agreement with the national control authorities.

Tests for identification cover both the antigenic activity and the nature of the antigen protein. The protein contaminants are determined by standardised SDS-PAGE gels under reduced and non-reduced conditions. Silver does not stain all proteins with the same intensity and is thus favoured for qualitative assessment only. The quantitative purity can be measured by Coomassie Blue staining. According to the WHO draft requirements, the protein purity should be at least 95%. Known standards must be run alongside the vaccine at a concentration of 1% of the total protein loaded onto the gel. The staining of these standards should be such that this amount is clearly visible.

The test for polysaccharides, DNA, and endotoxins must be developed by the manufacturer. Sterility is tested according to the requirements for classical bulk vaccines. As the antigen particle contains proteins and lipids, both components should be determined by reliable methods. The HBsAg content can be measured by commercial kits.

Most of the tests on the final vaccine are identical to those carried out on conventional vaccines: pH, volume of the filled container, aluminium and thiomersal content. Sterility, abnormal toxicity, and pyrogens are tested following the requirements of the WHO and the European Pharmacopoeia. The potency test is described by the WHO requirements. Dilutions of the vaccines to be tested and a reference vaccine are injected into groups of mice. The seroconversion rate of each group is determined and the relative potency of the vaccine calculated.

Vaccines are available as reference preparations, such as the proposed international reference preparation distributed by the National Institute for Biological Standards and Control (London). "In-house" working reference vaccines can be selected by using either a commercial licensed plasma vaccine or a homologous DNA preparation validated in clinical trials.

Another important issue is the stability of each vaccine lot. Experience with live attenuated vaccines has shown that it is very useful to carry out an accelerated stability test on each vaccine lot. The WHO guidelines for such vaccines now require this test. Vaccine samples of each lot are exposed at 37°C for 7 days. The relative potency of the exposed vaccine should not be significantly different from the value obtained with the non-exposed vaccine. This test, carried out on each lot, is the best guarantee of consistency.

Conclusion

"Classical" vaccines have been successfully produced and tested for several decades. The experience obtained in their quality assessment has been used as a basis and completed by "newer" analytical techniques developed for biomolecules obtained by recombinant DNA processes. It has been shown that the different guidelines and points-to-consider issued by national and supranational licensing and marketing authorities form a valid basis for the quality control of the "newer" vaccines. Hepatitis-B vaccine derived from transformed yeast cells meets in all respects the quality requirements and has been licensed world-wide. Extended clinical trials and experience in the field after administration of more than one million doses confirm the validity of the developed methods to assess the safety and the immunogenicity of the "new" hepatitis-B vaccine.

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