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Research Article

Molecular Genetic Analysis of the Strain Leningrad-16 Used for the Production of Measles Vaccine

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Abstract

Aim: To study the genetic stability of the measles virus strain Leningrad-16 (L-16) used for the production of vaccine at JSC NPO Mikrogen.

Materials and Methods: A series of production and sowing strains of L-16 (JSC NPO Mikrogen), ready-made series of measles vaccines from various manufacturers, and the strain of measles virus genotype D6 were studied. Molecular genetic study of the strains was performed using RT-PCR followed by restriction analysis and sequencing.

Results: The complete genome sequences of the production and sowing strains of L-16 that are used for vaccine production were obtained. The sequence of the vaccine strain was deposited in GenBank. Strain L-16 was confirmed to be genetically stable. The obtained data demonstrated the possibility of using the RT-PCR method with subsequent restriction analysis to confirm the authenticity of the vaccine strain L-16 in finished mono and three component vaccines.

Conclusion: The results of the study suggest the applicability of the molecular genetic methods to confirm the authenticity of the studied strains not only at the production stages, but also in the finished series of vaccines.

Keywords: *Measles Virus Strain Leningrad-16; Genetic Stability; Restriction Fragment Length Polymorphism Method.*

Introduction

Measles is a highly contagious disease characterized in the prodromal period by fever, cough, runny nose, conjunctivitis, followed by the appearance of a generalized maculopapular rash [1,2]. The measles virus that causes this disease belongs to the genus Morbillivirus, family Paramyxoviridae [1,2]. Based on the data on the nucleotide sequence, 24 genotypes of the measles virus are distinguished. Genotyping is carried out according to the nucleotide sequence of at least 450 bp encoding the COOH-terminal fragment of the nucleoprotein N protein [1,2]. The means of preventing the disease is the use of a live measles vaccine, for the production of which attenuated strains are used. Strains Mo ra-ten, AIK-C, Schwarz, Edmonston-Zagreb and Edmonston B were obtained from the original strain Edmonston, strains CAM-70, Shanghai-191, Changchun-47 and Lenin-grad-16 (L-16) were obtained independently [3-6]. At the same time, strains Changchun-47 and L-16 were passaged in different cell cultures, but they have a common "progenitor" - the Leningrad-4 strain. All of these vaccine strains belong to genotype A [2-6].

In Russia, the live measles culture vaccine is produced by JSC NPO Microgen and FBSI SSC VB Vector. Both manufacturers use the same strain, L-16. Modern requirements for the production of vaccine preparations containing live strains of viruses imply the control of the genetic stability of production strains, including strains of the measles virus (SMV), which is recommended by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (International Conference on the Harmonization of Technical Requirements for the Registration of Medicinal Products for Human Use) 1 and the State Pharmacopoeia of the Russian Federation2. All manufacturers, except for the Serum Institute of India (India), have made available information on the nucleotide structure of industrial ballasts and their genetic stability [3-6]. Information on the nucleotide structure of strains to control the authenticity of vaccine strains, not only at the production stages, but also in finished vaccine batches, allows the use of molecular genetic methods.

The State Pharmacopoeia of the Russian Federation regulates the use of a neutralization reaction to confirm the authenticity of a production ball valve, as well as production strains of mumps and rubella. For measles, mumps and rubella viruses, a high serological cross between strains is known - the immune serum obtained against the vaccine strain has a virus-neutralizing activity against other strains of the family [7-11]. Thus, the method of neutralizing a vaccine strain with a specific immune serum allows only to confirm the belonging of the investigated vaccine strain of the virus to the genus and family. On the contrary, the method of restriction fragment length polymorphism RFLP allows to confirm the authenticity of the particular vaccine strain, which was demonstrated for the Leningrad-3 mumps virus, the rubella virus strain RA-27/3 and ShVK Shanghai-191 [11-13]. The purpose of this work was to study the structure of the production and seed ShVK L-16 used by NPO Microgen for the production of monovalent and three-component vaccines, as well as to study the possibility of using molecular genetic methods to confirm the authenticity of the production strain in the finished product. vaccine form.

Materials and Methods

We used the measles virus genotype D6 (MV226, Mvi / Luxembourg LUX / 30/97/2), kindly provided by Dr. C.P. Muller [8]. The strain was used as a control during the restriction analysis of vaccine SCCs. We used vaccines from the following manufacturers:

1. JSC NPO Microgen:
    - production ShVK L-16 (series No. 10);
    - seeding ShVK L-16 (series No. 200);
    - combined vaccine against measles, rubella and mumps “Vaktrivir” (series M0015ek, M0020ek, M0021ek);
    - Cultural live attenuated rubella vaccine (one series);
    - Mumps vaccine (Leningrad-3 strain, one series);
    - Attenuated live measles vaccine (L-16 strain, three series);
2. “Serum Institute of India” - live attenuated vaccine against measles, mumps and rubella (four series);
3. “GlaxoSmithKlien Biologicals” - “Prio-Rix”, live attenuated vaccine against measles, mumps and rubella (three series);
4. “Merck and Co., Inc.” - ProQuad, measles, mumps, rubella and chickenpox vaccine (one batch).

To isolate RNA from the samples, we used the RIBO-sorb kit (AmpliSens, Russia), the extracted RNA was in a volume of 50 µl. When carrying out the reaction of reverse transcription (RT) and hydrolysis by restriction endonuclease, enzymes and components of reaction mixtures produced by NPO SibEnzyme (Russia) were used. Plasmid pUCL16 (NPO SibEnzyme, cat. No D19) served as a positive control

for RT-PCR and RFLP. The DNA plasmid pUCL16 is 3568 bp. The plasmid contains unique recognition sites for EcoR1, Sac1, Kpn1, Xma1, BamH1, Xba1, Sal1, HincII, Acc1, Pst1, BspM1, Sph1, and HindIII located in the polylinker. The plasmid contains an insert of the cDNA fragment of the measles virus, strain L-16, 882 bp long, corresponding to positions 1466-2324 bp. in the genome of the measles virus. The fragment has restriction sites with restriction enzymes BstSFI and CciI.

The RT reaction was carried out under the conditions described in detail earlier [12]. Polymerase chain reaction (PCR) was carried out in the same tubes as RT, since all the components necessary for PCR were already contained in solutions, and the template was cDNA produced from viral RNA. The thermal cycling mode fully corresponded to the one described earlier [12].

After PCR, 5 μL of restriction buffer "ROSE" (cat.no.B021) and 20 μL of water were added to 25 μL of the resulting mixture, then divided into 2 aliquots of 25 μL and 1 μL was added to one of them. restriction enzymes CciI. After incubation for 1 h at 37 ° C, the samples were loaded onto a 1.5% agarose gel and electrophoresis was performed in TAE buffer. To determine the complete sequences of the production strain and the seed ShVK L-16 (JSC NPO Microgen), primers were synthesized that completely overlapped the sequence of the measles virus genome with a step of 500 bp. [14].

Sequencing was performed on a Prism 310 Genetic Analyzer using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing data were analyzed using the Chromas 2.22 software (Technelysium Pty Ltd.).

Results

Study of the primary structure of the full-size genomes of the production and seed ShVK L-16

Production ShVK L-16 (series No 10, prepared in 1993) passed 21 passages in a primary culture of guinea pig kidney cells and 4 passages in a primary culture of Japanese quail fibroblasts. The seed ShVK L-16 (series No. 200, prepared in 2003) underwent 21 passages in a primary culture of guinea pig kidney cells and 6 passages in a primary culture of Japanese quail fibroblasts. In the process of producing a ready-made series of vaccines, the inoculum virus undergoes 1 passage in the culture of Japanese quail fibroblasts.

The cDNA sequencing results of the production strain are presented in GenBank (# JF727649). Comparative sequencing results of the seed virus cDNA and the cDNA obtained from the finished vaccine batch (GenBank JF727650) prepared from the indicated seed virus indicate that the nucleotide sequences are identical to those of the production strain. This suggests that the strain L-16 of JSC NPO Microgen is genetically stable at the stages of vaccine production.

Selection of a fragment of ShVK L-16 suitable for confirming the authenticity of the strain by RFLP

For ShVK L-16, the selection of fragments for further use of RFLP was carried out in accordance with the criteria described earlier [12]. For analysis, reference sequences of all 24 measles virus genomes and all vaccine strains were selected from the GenBank database. As the first step of the analysis, we compared the reference sequences for the COOH-terminal fragment of the nucleoprotein N position 1126-1575 nt; area used for genotyping. Analysis of multiple alignment shows that the reference sequence of the genotype A of measles virus differs from the reference sequences of other genotypes at position 1547 (nucleotide residue T, while in other sequences - G). The T nucleotide residue is part of the BstSFI (CTATAG) restriction enzyme recognition site, which makes it possible to use RFLP to differentiate genotype A from other genotypes in one step. Of the 48 full-genome sequences of the measles virus genotype A presented in GenBank, 43 contain the BstSFI restriction endonuclease recognition site in the form of the CTATAG sequence. Among the vaccine strains, only Shanghai-191 does not contain this site.

The BLAST analysis did not reveal the presence of this restriction enzyme site in this region of the genome in strains belonging to other genotypes. Thus, the RFLP method using the BstSFI restriction enzyme (SfeI) can make it possible with a high degree of accuracy

(~ 90%) to determine the belonging of a strain to genotype A. For the analysis of nucleotide sequences, the complete genome sequences of vaccine strains of measles virus were selected from the GenBank database: AIK-C (AF266286, AB046218), Moraten (AF266287), Morten (FJ211583), Schwartz (FJ211589), Edmonston AY4883), CAM-70 (DQ345722, DQ345721), Changchun-47 (FJ416068), Shanghai (FJ416067), L-16 (JF727650, JF727649). Multiple alignment of the sequences of vaccine strains and the reference strain of genotype A Edmonston in order to identify nucleotide substitutions inherent only in ShVK L-16, showed that this strain has unique substitutions (table). Two of the 4 unique substitutions form restriction endonuclease sites (underlined in the table) that can be used to differentiate the L-16 ShVK using RFLP. These are the TCATGA (CciI) and CATG (FatI and FaeI) sites near position 2143, as well as the AGCT site (AluI and AluBI) near position 7190. For the hydrolysis of cDNA, it seems convenient to use the larger-cleaved restriction enzyme CciI.

BLAST analysis of the fragment that contains the TCATGA site in the L-16 ShVK showed that this site is not contained in any of the published genomes of other ShVKs, both belonging to gene type A (including all vaccine strains) and other genotypes. Thus, this site can serve as a unique marker for this vaccine strain.

Selection of primers for amplification of the genome region with the TCATGA site at position 2143, calculation of the lengths of the fragments formed during the hydrolysis of the PCR product

To amplify the fragment containing the TCATGA site, primers were selected to amplify the 378 bp fragment: L1970f 5 'CAG GCA GTT CGG GTC TCA GCA 3'; L2324r 5 'CAG AAG CCC TGA ACC CCA TAG AGA 3'. As a result of PCR using these primers, a DNA fragment of 378 bp will be amplified containing the CciI restriction site, which is present only in the L-16 strain. After treatment with restriction endonuclease CciI, this fragment (in the case of using cDNA of strain L-16 in the reaction) decomposes into 2 fragments with lengths of 175 and 203 bp. As a positive control during the reaction, it is possible to use the pUCL16 DNA plasmid (NPO SibEnzyme).

When using the above primers, a 378 bp fragment will be obtained. When the fragment is hydrolyzed with restriction enzyme CciI, fragments of 175 and 203 bp in length will be obtained. When primers are used to amplify a fragment for genotyping [2,14], a 481 bp fragment will be obtained. with a restriction site with a restriction enzyme BstSFI. When the fragment is hydrolyzed with the restriction enzyme BstSFI, fragments of 188 and 293 bp in length will be obtained (Figure 1).

Results of RT-PCR followed by restriction with restriction endonuclease CciI in samples containing SCC

To isolate RNA and then carry out RT-PCR with restriction, we used SCC and samples of ready-made vaccine forms from various manufacturers. The result of RT-PCR followed by restriction is shown in figure 2. By amplification of the inoculum and the strain, an amplicon with an assumed calculated length of 378 bp was obtained from the finished form of the vaccine. Restriction of the resulting amplicon with restriction endonuclease CciI yielded 2 fragments with calculated lengths of 175 and 203 bp.

When carrying out RT-PCR followed by restriction of genotype D6 ShVCs, ready-made forms of vaccines from different manufacturers containing different ShVKs, an amplicon of the calculated length was obtained, but the result of its restriction with restriction endonuclease CciI is negative: fragments with the calculated lengths of 175 and 203 bp. not received (Figure 2). This is due to the fact that strains of other genotypes of measles virus and ShVK used by other manufacturers do not contain a Ccil restriction site in the genome fragment from 1970 to 2324 bp. When carried out under the same conditions RT-PCR followed by restriction of vaccines against mumps and rubella produced by JSC NPO Microgen, a fragment of 378 bp in length. was not amplified, i.e. the result was negative.

Thus, the selected primers make it possible to carry out RT-PCR of all RNA isolated from samples containing the measles virus and obtain a 378 bp amplikon (Figure 2). Only those fragments that contain a restriction site for the CciI restriction enzyme, which is characteristic of the L-16 ShVK, allow one to obtain 2 fragments of 175 and 203 bp in length during restriction. The fragment of ShVK L-16 proposed for amplification with subsequent restriction allows confirming the authenticity of the strain not only at the production stages, but also in the finished form of the vaccine.

Application of selected primers for amplification of a 378 bp fragment. and restriction endon clease CciI makes it possible to distinguish the L-16 ShVK from the ShVK used in the production of other vaccines using RFLP.

Conclusion

ShVK L-16 for measles vaccine production is genetically stable at all stages of production. Molecular genetic control methods are more specific for confirming the authenticity of vaccine strains. The RFLP method is also applicable to confirm the authenticity of ShVK L-16 and can be used not only at the stages of vaccine production, but also in the finished form of the drug - both in mono- and combined vaccines.

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