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Progress on the research and development of inactivated EV71 whole-virus vaccines

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The prevalence of diseases caused by EV71 infection has become a serious public health problem in the Western Pacific region. Due to a lack of effective treatment options, controlling EV71 epidemics has mainly focused on the research and development (R&D) of EV71 vaccines. Thus far, five organizations have completed pre-clinical studies focused on the development of inactivated EV71 whole-virus vaccines, including vaccine strain screening, process optimization, safety and immunogenicity evaluation, and are in different stages of clinical trials. Among these organizations, three companies in Mainland China [Beijing Vigoo Biological Co., Ltd. (Vigoo), Sinovac Biotech Ltd. (Sinovac) and Institute of Medical Biology, Chinese Academy of Medical Science (CAMS)] have recently completed Phase III trials for the vaccines they developed. In addition, the other two vaccines, developed by National Health Research Institutes (NHRI) of Taiwan and Inviragen Pte., Ltd (Inviragen), of Singapore, have also completed Phase I clinical trials. Published clinical trial results indicate that the inactivated EV71 vaccines have good safety and immunogenicity in the target population (infants) and confer a relatively high rate of protection against EV71 infection-related diseases. The results of clinical trials suggest a promising future for the clinical use of EV71 vaccines. Here, we review and highlight the recent progress on the R&D of inactivated EV71 whole-virus vaccines.

Keywords: enterovirus 71, hand-foot-mouth diseases (HFMD), inactivated whole-virus vaccine, immunogenicity, protective effect, standard

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Introduction

The first EV71 case was reported in the United States in 1969.[1] In recent years, there has been a continually increasing trend of EV71 infection-related epidemics in the Western Pacific region.[2] To effectively control the prevalence of diseases caused by EV71 infection, different types of EV71 vaccine candidates are under active R&D, including inactivated whole-virus vaccines,[3-8] live attenuated vaccines,[9,10] recombinant vaccines[9,11-14] and peptide vaccines;[15,16] among these approaches, research on inactivated whole-virus vaccines has shown rapid progress due to the advantages of immunogenicity and mature manufacturing technologies.[3,4,6,17,18] Good immunogenicity and preventive effects were demonstrated for several inactivated vaccines in animals.[5-8] In order to promote the R&D process of inactivated EV71 vaccines, a series of studies on quality control standards and standard references were performed, leading to the establishment of a quality control and evaluation system for inactivated EV71 vaccines that laid a solid foundation for clinical research and approval of new vaccines.[19-23] At present, five organizations, located in Mainland China, Taiwan and Singapore, have reported rapid progress on the development of inactivated EV71 vaccines. Phase I and II clinical trial results have shown that the inactivated vaccines have good safety and immunogenicity.[4,18,24] Recently published Phase III reports showed that the EV71 vaccines developed by three enterprises in Mainland China all have good safety and

mediate protective effects against EV71 infection-related diseases.[25,26] In this article, the R&D of inactivated EV71 vaccines, relevant quality control standards and the perspectives of EV71 vaccine research are summarized.

R&D Progress of Inactivated EV71 Vaccines

R&D progress of inactivated EV71 vaccines in Mainland China. In the R&D process of inactivated EV71 vaccines, Vigoo, Sinovac and CAMS established their respective C4 genotype vaccine strains, the seed lot system and cell banks of Vero or diploid cells according to WHO guidelines and the current edition of the Chinese Pharmacopoeia and Technical Guideline for Pre-Clinical Research of Preventive Vaccines issued by the State Food and Drug Administration, PR China (SFDA). They also completed cross-protection studies of the vaccine strains and conducted research on process optimization, animal immunogenicity and protective effects in animal models.[5-8,27-31] All three vaccine candidates entered clinical trials between December 2010 and February 2011. In Phase I and II trials, the doses of the EV71 vaccine developed by Vigoo were 160, 320 and 640 U/dose (0.25, 0.5, 1.0 μg protein/dose), while the Phase III dose was 320 U/dose. Phase I and II doses of the EV71 vaccine developed by Sinovac were 100, 200 and 400 KU/dose, while the Phase III dose was 400 U/dose. The Phase I and II doses of the vaccine developed by CAMS were 160, 320 and 640 EU/dose, while the Phase III dose was 100 U/dose. Sinovac was the first to publish its Phase I clinical trial results, which indicated that the vaccine was safe and elicited protective antibody responses in adults, children and infants.[18] Subsequently, Vigoo reported its Phase I trial results, confirming that the vaccine has good safety and immunogenicity in the target populations of adults, children and infants.[24,32] The Phase II clinical trial results reported by Vigoo suggest that 320 U is the optimal dose for EV71 vaccination; while their vaccine were not found interference with immune response due to poliovirus vaccination induced crossimmunity, and the neutralizing antibody titer against EV71 significantly declined at month 8 compared with day 56, suggesting a need for a booster shot.[33] The EV71 vaccine developed by CAMS using diploid cells has a clinical safety and immunogenicity profile consistent with the other two vaccines developed using Vero cells as a substrate.[4] Taken together, the results of Phase I and II clinical trials demonstrate that the three inactivated EV71 whole-virus vaccines have good safety and immunogenicity in humans, especially children and infants. Moreover, the three companies recently announced the results of Phase III clinical trials on vaccine safety and the protection rate against EV71 infection-related diseases. In each trial, about 10000 healthy infants and young children were immunized with an EV71 vaccine following the d0/d28 two-vaccination procedure, and the protective effect was observed in the following year (two epidemiological seasons). The report by Sinovac showed a protection rate of 95%,[26] while the vaccine developed by CAMS showed a protection rate of 95% or more for the diseases caused by EV71 infection.[34] A study by Vigoo also demonstrated that the vaccine has similarly good safety and efficacy profiles.[35] The three vaccine clinical trials all showed good protective effects against EV71 infection-related diseases, which will advance the vaccine evaluation and licensing process.

R&D progress of inactivated EV71 vaccines by the NHRI of Taiwan. NHRI employed a B4 genotype vaccine strain[36] and established the Vero cell bank in accordance with the relevant requirements of the FDA.[17,36] Based on a comparative study of the vaccine strains,[37,38] research with regards to process optimization,[39,40] serum-free culture techniques for inactivated vaccines,[41] quality control methods[20,21] and immunogenicity assays[38] has been conducted. The vaccine entered clinical trials in 2010, and the doses employed in the clinical trials were 5 μg and 10 μg protein/dose. At present, Phase I clinical trials have been completed, and the result showed that the EV71 vaccine can induce a 4-fold or greater increase in neutralization antibody titers (NT) in healthy adults after only the first vaccination.[42]

R&D progress of inactivated EV71 vaccines by Inviragen of Singapore. The vaccine developed by Inviragen using a B3 genotype vaccine strain and Vero cells as the substrate entered clinical trials in 2011. High-dose and low-dose groups were included in the clinical trials, and Phase I trials have been completed.[43]

Research on Vaccine Quality Control Standards and Reference Standard Preparation

The establishment of vaccine quality control standards and reference standards are major steps in vaccine development and evaluation. Three EV71 vaccine candidates began registered clinical trials around the same time. In order to facilitate comparisons of the quality and efficacy of EV71 vaccines, the Chinese Drug Administration unified the quality control requirements for EV71 vaccines. Specifically, the establishment of vaccine strains, seed lot system and cell banks must meet the requirements of the Chinese Pharmacopoeia and WHO procedures, which mandate that the VP1 nucleotide sequence in the major protective area must remain unchanged from the primary seed lots to the working seed lots to ensure genetic stability. Among the EV71 vaccines that have entered into clinical trials, four of them have used Vero cells as the substrate for production, so the purity of the vaccine was key point for quality control. The content of EV71 antigen should not be less than 95.0% using the HPLC method, residual cell-substrate DNA should be less than 100 pg/dose and host contaminating proteins should be no more than 10% of the total protein content.

Research has shown that while Vero cells can induce tumors after more than 170 passages, such tumorigenicity was not found in passages 134–150.[44,45] Currently, Vero cells have been approved for IPV and rotavirus vaccine production. Several live viral vaccines produced in Vero cells have been licensed in the United States over the last decade: the smallpox vaccine (2007) and two rotavirus vaccines (2006 and 2008).[46] The WHO recommends that vaccine production organizations use 150 passages as qualified passage as far as

possible. In addition, WHO recommends that the final DNA content per dose of the vaccine product is less than 10 ng.[47] The US FDA mandates that the residual DNA content of each dose of biological products be no more than 100 pg. The Chinese Pharmacopoeia (third edition, 2010) requires the residual exogenous DNA in Vero cell culture-derived vaccines be no more than 100 pg per dose. The IPV vaccine manufactured by Pasteur Co., Ltd, using Vero cells requires residual DNA content to be below 10 pg.[48] For the two EV71 vaccine candidates developed by Mainland Chinese companies using Vero cells as the substrate, cell passages were kept below 150, which is in line with the WHO requirement, and residual DNA was kept less than 100 pg/dose. Taking into account that the EV71 vaccine target populations are mainly healthy infants and young children, the residual DNA standard should be further improved to 10 pg/dose. It is very important to accurately measure the residual cellular DNA content in EV71 vaccine products. The National Institutes for Food and Drug Control (NIFDC) has established the first batch of Vero cell DNA reference standards (Approval No: 2011-NRS0044). The successful establishment of a Vero cell DNA quantitative reference will facilitate the standardization of dot blot hybridization for measuring residual host cell DNA contamination.[49]

The detection of vaccine antigens and neutralizing antibodies is important for vaccine development. To enable accurate detection of antigen content and the level of neutralizing antibodies, a series of studies were performed in China. CAMS and Sinovac developed reagents for quantitative detection of EV71 vaccine antigen.[22,23] A collaborative study on determination method of EV71 neutralizing antibody was performed by NIFDC.[50] NIFDC also developed a national EV71 vaccine antigen reference standard and neutralizing antibody standards (Approval No: 20100023, 0024).[19] The vaccine antigen standard has a set value of 1600 U/ml, while the neutralizing antibody standards include 3 references: strongly positive (N12: 1000 U/ml), weakly positive (N3) and negative (J10), and these are used for vaccine quality control, vaccine dosage determination, immunogenicity and efficacy evaluation. Establishment of national EV71 antigen and neutralizing antibody quantitative standards facilitates vaccine quality control and comparative analysis of the immunogenicity of vaccines developed by Mainland Chinese enterprises, while also promoting the EV71 vaccine development process. However, the epidemic EV71 strain varies among different countries and regions while the dominant strain in Mainland China since 1998 has maintained a C4 genotype, so determining whether these standards are representative and appropriate as the global antigen and neutralizing antibody standards deserves further investigation.

In order to provide sufficient data on immunogenicity and antibody-response dynamic characteristics for clinical trials, Mao unified the units of dose (U/ml) of the three vaccines from Mainland China with the antigen reference standard and compared their immunogenicity in mice.[51] The results showed that despite the differences in vaccine strains used, the cell substrates used, the production process employed and the differences in immunogenicity existing between the vaccine strains and original extracts, the final aluminum-adjuvant vaccines have similar immunogenicity.[51] This result was consistent with the results of human clinical trials,[18,24] suggesting that the immunogenicity of vaccines prepared from different strains, cell substrates and production processes can be compared on the basis of a unified dose. In order to solve the shortcomings of traditional neutralizing antibody assays, such as being labor-intensive, subjective and time-consuming, NIFDC has developed a pseudovirus-luciferase assay for rapid and quantitative detection of EV71 neutralizing antibody (NTAB) levels after vaccination.[52]

NHRI investigated the methodology for quantifying EV71 antigens (VP2-based Q-ELISA) to develop surrogate markers for vaccine efficacy studies. The Q-ELISA method to detect the EV71 VP2-28 antigen (residues 136–150 of VP2) has been developed, and the test results demonstrated a dose-response relationship with vaccine immunogenicity.[20] In addition, they also completed the development and qualification of an in-house standardized virus neutralization assay (RD cell micro-neutralization assay). The structure and immunogenicity of empty particles (E-particles) and full particles (F-particles) prepared by different processes were compared in order to guarantee the vaccine efficacy by establishing corresponding quality control standards for the manufacturing process.[21]

Future Perspective of EV71 Vaccine Research

After evaluation of vaccine safety, immunogenicity and protective effects, future efforts should focus on international vaccine applicability, strengthening the monitoring of prevalent strains and variation, development of EV71/CA16 combination vaccines and promoting international cooperation for establishment of global vaccine standards.

The applicability of EV71 vaccines. EV71 has one serotype and three genotypes, which can be further divided into 11 genetic subtypes, including A, B1-B5 and C1-C5. Gene mutation and recombination frequently occur during EV71 epidemics, with the exception of those occurring Mainland China, where the dominant epidemic strain has remained C4 since 1998.[53] The common epidemic strains observed after 2000 are C2, C4, C5, B4 and B5. Recent studies have discovered new genotypes, and this poses challenges in the selection and application of vaccine strains (i.e., the applicability of the vaccines).[54] Thus, due to the presence of different genotypes, subtypes, and potentially emerging new genotypes, current R&D should focus on the cross-protection elicited by current vaccines against EV71 strains of subgenotype C4, B3 or B4 after the safety, immunogenicity and protective effects of these vaccines are obtained. Our recent research using serum from vaccine-immunized populations suggests that the single C4-strain derived vaccine confers a high level of cross-protection [unpublished data].

The need for the development of EV71/CA16 combination vaccines. CA16 is one of the main pathogens causing HFMD. Although the number of severe cases and deaths caused by CA16 is lower than caused by EV71, the clinical

symptoms of EV71 and CA16 infections are difficult to distinguish. There is an urgent need to develop CA16-specific or combined CA16/EV71 vaccines.[17,38,55] Several enterprises in Mainland China have completed pre-clinical studies on EV71/CA16 combination vaccines. Animal experiments showed that the bivalent vaccine could elicit a neutralizing antibody response to a similar extent as monovalent vaccines [unpublished data].

The international demand for EV71 vaccine development. The recently announced protective effects of EV71 vaccines in clinical trials highlight the promising potential of these vaccines as a new vaccine at the stage closest to approval and application. In the past, R&D as well as the promotion of new vaccines (e.g., HPV, rotavirus and pneumonia vaccines) has been dominated by large international companies such as Merck Sharp and Dohme (MSD) or GlaxoSmithKline (GSK). These companies are well experienced in the development of quality control standards for the R&D of new vaccines, and have strong capabilities in carrying out international clinical trials and application of new vaccines. However, the EV71 vaccine is a new vaccine led by developing countries, which have limited experience in establishing international reference standards. Therefore, successful R&D and promotion of vaccines to control EV71 epidemics requires cooperation between governments and vaccine manufacturers to be strengthened.[56] In the meantime, international standardization of vaccine quality control should be performed under the guidance of the WHO.[55] Recently, the WHO ECBS approved a joint research project between the NIFDC and National Institute for Biological Standards and Control (NIBSC) to develop EV71 vaccine quantitative standards for detection of neutralizing antibodies and antigens, which are expected to help promote the internationalization of the EV71 vaccine development.

Conclusion

The development of EV71 vaccines will play an important role in controlling EV71-related epidemics. In addition to the evaluation of the safety, immunogenicity and protective effects of the inactivated vaccines, future research should also focus on the cross-protective effects of vaccines developed from strains of different genotypes, strengthening the monitoring of prevalent strains and strain variation, development of EV71/CA16 combination vaccines and promoting international cooperation for establishment of global vaccine standards. In particular, the WHO-guided formulation of vaccine quality control standards and international cooperation for establishment of reference standards will greatly promote the R&D and application of EV71 vaccines worldwide.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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