Progress on the research and development of EV71 vaccine

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Background

Since the first Enterovirus 71 (EV71) case was reported in the United States in 1969[1], EV71 has emerged as a significant cause of hand, foot and mouth disease (HFMD), herpangina, encephalitis, aseptic meningitis, cerebellar ataxia, poliomyelitis like syndrome and even fatal disease.

EV71 belongs to Picornaviridae, a family of single-stranded positive-sense RNA viruses. The EV71 genome encodes a long polyprotein with a single open reading frame. The single polyprotein can be divided into three different regions (P1, P2 and P3). The P1 region is the structural proteins VP1–VP4. The P2 and P3 regions encode the non-structural proteins: 2A, 2B, 2C, 3A, 3B, 3C and 3D [2]. EV71 has only one serotype, but it can be further classified into three genogroups on the basis of VP1 sequence: A, B and C, and can be further divided into 11 genotypes (A, B1–B5 and C1–C5) [3].

EV71 caused significant public health problems. In 1997, the large outbreak of EV71 in Malaysia heralded series of outbreaks across the Asia-Pacific region. In 1998, the largest EV71 epidemic outbreak in Taiwan: an estimated 1.5 million people were infected and more than 405 children were admitted to hospital for serious neurological complications, of whom 78 died [4]. The latest large Asian-Pacific epidemic was in China in 2008, when around 490,000 infections and 126 deaths in children were reported [5]. In addition, many areas including Vietnam, Singapore, Japan and Malaysia, have experienced low level and sporadic cases that occur every 2-3 years [6].

Given the public health significance of EV71 and the lack of effective antiviral therapies, vaccines have become a top priority in EV71 control strategies. Several EV71 vaccine candidates have been evaluated in animals and clinical trials.

R&D of EV71 Vaccine

1. Live attenuated vaccines

An example of successful attenuated vaccine is poliovirus and has been approved for worldwide application. Based on the similarities between poliovirus and EV71,
a live attenuated strain of EV71 (S1-3’) from genotype A has been studied in monkeys [7]. Three cynomolgus monkeys were intravenously inoculated with the attenuated virus, followed by lethal challenge with the parental virulent strain EV71 (BrCr-TR). Monkeys inoculated with EV71 (S1-3’) showed a mild neurological symptom (tremor) but survived lethal challenge by virulent EV71 (BrCr-TR) without exacerbation of the symptom. The immunized monkey sera showed a broad spectrum of neutralizing activity against different genotypes of EV71, including genotypes A, B1, B4, C2, and C4. Although these results demonstrate promise for a live attenuated vaccine against EV71, this vaccine did demonstrate attenuated neuro-virulence, as evidenced by mild neurological symptoms and isolation of virus from the spinal cord, indicating that the vaccine itself was not completely attenuated, and the further work on attenuation is needed.

2. Inactivated vaccine

Among the various vaccine candidates, inactivated whole-virus vaccine are in some ways the most ready to develop further, because the principles of vaccines based on inactive whole-virus are well established.

In the animal level studies conducted by Wu et al. and Yu et al., protection against lethal EV71 infection by passive transfer of maternal antibodies from heat-inactivated or formalin-inactivated vaccine immunized adult mice has been observed in neonatal mice. Meanwhile, maternal immunization with inactivated EV71 vaccine was able to prolong the survival of suckling mice after EV71 lethal challenge. These results suggest that inactivated EV71 vaccines are capable of eliciting broadly neutralizing antibodies and protection against viral challenge in mice [8,9].

Based on historical experiences with the animal studies mentioned above, the inactivated whole-virus vaccines are very feasible and could be licensed readily. Until recently, the clinical trials have been conducted by five organizations in Asia (Table 1). Three organizations in China have finished Phase III clinical trials, and the results show very similar good safety and protective efficacy against EV71 infection and related disease. At present, the Phase I clinical trials have been completed by NHRI of Taiwan, the immunogenicity data showed that 90% of vaccine recipients have a 4-fold increase in neutralization antibody titers (NT) after only the first vaccination. The seroconversion rate on day 42 was 93.1%. The high and low dose groups were introduced into the Phase I clinical trials by Inviragen of Singapore, the detail results are waiting for published.

3. Virus-like particle (VLP) vaccines

VLP vaccine could be produced by co-expression of the P1 region and viral
### Table 1: Inactivated Whole-virus vaccine candidates finished clinical trials

<table>
<thead>
<tr>
<th>Organization</th>
<th>Genotype</th>
<th>Cell line</th>
<th>Finished clinical trials</th>
<th>Dosage (adjuvant)</th>
<th>Vaccination times</th>
<th>Protection rate (Phase III)</th>
<th>Reference.</th>
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</table>
| Vigoo        | C4       | Vero      | Phase I,II,III           | Phase I and II: 160, 320 and 640 U/dose ( alum)  
Phase III: 320 U/dose ( alum)  
Phase III: 320 U/dose ( alum) | Two doses: day 0 and 28 | 90.5% | [10-12] |
| Sinovac      | C4       | Vero      | Phase I,II,III           | Phase I and II: Adults and children: 200 and 400 U/dose;  
Infants: 100, 200 and 400 KU/dose ( alum)  
Phase III: 400 U/dose ( alum) | adults: day 0, 14 and 28; Children and infants: day 0, 28 and 56; | 95% | [13-15] |
| CAMS         | C4       | Human diploid cells (KMB-17) | Phase I,II,III | Phase I and II: 160, 320 and 640 EU/dose ( alum)  
Phase III: 100 U/dose ( alum)  
Phase III: 100 U/dose ( alum) | Two doses: day 0 and 28 | 95% | [15, 16] |
| NHRI         | B4       | Vero      | Phase I                  | Phase I: 10 and 20 μ g/dose ( alum) | Two doses: day 0 and 21 | No | [17] |
| Inviragen    | B3       | Vero      | Phase I                  | Phase I: high and low dose ( alum) | Two doses: day 0 and 28 | No | [15] |

Note: Vigoo: Beijing Vigoo Biological Co., Ltd., China; Sinovac: Sinovac Biotech Co., Ltd., China; CAMS: Institute of Medical Biology, Chinese Academy of Medical Biology, China; NHRI: National Health Research Institutes, Taiwan, China; Inviragen: Inviragen Pte., Ltd.
protease 3CD in the SF9 or yeast cells \cite{18, 19}. More importantly, the VLP immunization of female mice conferred protection to neonatal mice against lethal (1000LD50) EV71 challenge, survival rate was 89%. Compared with the VLP immunization, vaccination with denatured VLP and heat-inactivated EV71 elicited lower neutralization titers and conferred less effective protection to newborn mice \cite{20}. These data collectively indicate the importance of preserving the conformation-dependent epitopes and the potential of VLP as a vaccine to prevent EV71 infection.

4. Recombinant protein vaccines

Wu et al. immunized the female mice with recombinant VP1 protein expressed by E. coli, then challenged the neonatal mice with EV71 (2.3×102LD50), the protection rate was 95.6% (22/23) \cite{8}. Recombinant VP1 protein vaccines have also been tested by oral delivery approaches. After oral immunization of transgenic tomato fruit expressing VP1 protein, serum from the immunized mice could neutralize the infection of EV71 to rhabdomyosarcoma cells. Moreover, the proliferation of spleen cells provided further evidence of both humoral and cellular immunity \cite{21}. In another research, mouse pups that received transgenic parental mice's VP1-milk orally, demonstrated relatively better health conditions after challenge with the respective virus as compared with the non-transgenic milk fed group. According to the serum-neutralization assay and serum antibody detection, the littermates suckling VP1-milk generated antibodies specific to EV71 \cite{22}.

5. Viral and bacterial vector-based vaccines

Using live viral or bacterial vectors to deliver antigen is another potential approach for EV71 vaccine development. Results for protection were reported with live Bifidobacterium or attenuated Salmonella-based VP1 subunit vaccine \cite{23, 24}. Varma et al. and Balraj et al. showed that orally immunize the mice with VP1 protein using live lactococcus lactis or recombinant baculovirus as carrier could induced humoral and mucosal immune responses against EV71 \cite{25, 26}.

This vaccination strategy appeared, at least in mice, to work successfully for preventing EV71 infection. Potential problems associated with this strategy, including lack of appropriate animal models and require more consideration of safety due to the main targeted population is the pediatric population.

6. DNA vaccine

Tung et al. evaluated immunogenicity of VP1 DNA and found that the VP1 DNA vaccine candidate could induce serum neutralizing antibody in mice \cite{27}. Another DNA vaccine developed by Wu et al., elicited a high neutralization titer and the protected rate against virus challenge (2.3×10^2LD_{50}) was 38% (10/26).
Prospects

As EV71 is highly contagious and causes life-threatening infections in Asian children, the development of EV71 vaccines will play an important role in controlling EV71-related epidemics. The results of clinical trials suggest a promising future for the clinical use inactivated whole-virus EV71 vaccine and will be the first EV71 vaccine in use.

Further research should also focus on development of EV71/CVA16 bivalent vaccine for HFMD and the consistence and systemic enterovirus surveillance, especially in endemic regions and countries are also needed.

Reference


