



Review Article

Hepatitis E Vaccine: Present and Future

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Abstract

Viral hepatitis is a major public health problem in the world, and it can be caused by blood- and food-borne viruses. Blood-borne hepatitis agents are HBV, HCV and HDV, whereas HAV and HEV are food-borne hepatitis viruses. HEV infection is an important infectious agent in developing countries, but it is also an emerging disease in developed countries, which is likely due to travel or immigration from endemic areas.¹ The main route of human HEV transmission is fecal-oral (fecally contaminated water),² although other routes were also reported such as person-to-person transmission,³ blood products,⁴ mother-to-child transmission⁵ and zoonotic transmission (e.g., by pigs, particularly in developed countries,⁶ and seafood⁷. Epidemiologically, only one serotype of HEV exists in the world. Genetically, the virus has been classified into four genotypes and several subgenotypes designated 1 (1a-e), 2 (2a and b), 3 (3a-j) and 4 (4a-g).⁸ Each genotype shows a distinct geographical distribution. Genotype 1 of HEV is reported from developing countries in Asia and Africa; genotype 2 has been detected in some countries in Africa as well as in Mexico; genotype 3 is distributed globally and genotype 4 of HEV is only found in Asian countries.⁹ The genotypes may not only vary with respect to their geographical distribution, but also in their pathogenicity. Genotypes 1 and 2 are primarily human pathogens, causing acute hepatitis in young, nonimmunocompromised people; genotypes 3 and 4, however, have been found in swine and other animals and could therefore be responsible for zoonotic transmissions, preferentially in the elderly or in immunocompromised patients.²

TAJ 2009; 22(2): 330-336

Introduction

Human HEV was initially identified by electron microscopy from the feces of infected patients and could be cloned in the early 1990s.² According to the eighth report of the International Committee on the Taxonomy of Viruses, HEV belongs to a separated family and genus named *Hepeviridae* and *Hepevirus*, respectively.¹⁰ HEV is an icosahedral, round nonenveloped virus (27-34

nm), with a single-stranded polyadenylated (+)RNA of approximately 7.5 kb in length. The virus consists of three open-reading frames (ORFs) comprising nonstructural and structural proteins, as well as two conserved nontranslated regions at the 5' and 3' termini of the genome. ORF1 encodes a nonstructural multifunctional polyprotein that the viral RNA-dependent RNA polymerase synthesizes. The virus major capsid

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protein, the main structural protein of HEV, is encoded by ORF2. The last and the smallest ORF3, which overlaps ORF1 (slightly) and ORF2 (mostly), encodes a small protein with unknown function, possibly mediating interactions between virus and host; for example, anchoring of the virus with the cytoskeleton.

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Clinical Features of HEV Infection

Clinical symptoms of HEV infection may range from asymptomatic (subclinical) to acute and fulminant hepatitis, presenting with usual clinical features of acute hepatitis as jaundice, splenomegaly, elevated liver enzymes (aspartate and alanine aminotransferase activities), bilirubinuria, which may go together with fever and vomiting, and also (rarely) with pancreatitis.¹¹ Acute infection usually leads to a self-limited hepatitis, with anti-HEV IgM appearing in the serum of infected persons within 2 weeks to 3 months. Among immunocompetent individuals, HEV infection is associated with a case-fatality rate of less than 0.1%.² However, this rate is much

higher in immunocompromised individuals and markedly elevated in pregnant women, especially in the third trimester, in which HEV-related mortality may reach up to 39-73%.¹² A recent, large study from India highlighted HEV as the major cause of acute hepatitis among pregnant women in India, and that HEV infection represented a significant risk for maternal mortality, obstetric complications and the fetal outcome.¹³ Similar observations were reported in an HEV infection outbreak among Sudanese pregnant women.¹⁴ Nevertheless, some studies, for example, from Egypt and southern India, did not report HEV-related fulminant hepatitis, despite a high prevalence of anti-HEV in pregnant women, and it is currently unclear which factors (e.g., genotype, time of infection or early vertical transmission in childhood) account for these differences¹⁵

Chronic HEV Infection: Myth or Underestimated Problem?

The concept of HEV causing only acute, self-limiting hepatitis has recently been challenged, as some reports suggest that HEV could be responsible for chronic hepatitis in organ transplant patients.¹⁶ In a cohort of 217 organ transplant patients presenting with elevated liver enzymes, Kamar *et al.* identified a total of 14 (6.5%) that had detectable serum HEV RNA.¹⁷ Among these patients, six resolved HEV infection, but eight showed persistently elevated liver enzymes and detectable serum HEV RNA for a mean observation period of 15 months, suggesting a chronic course of HEV infection.¹⁷ At present, it remains unclear whether HEV can truly cause chronic hepatitis. To date, persistent HEV levels have only been detected after organ transplantation in patients that were severely immunosuppressed, and a causative relationship between HEV persistence and ALT elevation/hepatitis has not been established. Furthermore, all cases of persistent HEV viremia were related to HEV genotype 3, indicating that this could represent a specific, atypical, opportunistic zoonotic infection in immunocompromised patients.¹⁸

Is a HEV Vaccine Needed?

From a clinician's point of view, a HEV vaccine would be highly desirable. Although the course of disease is benign in the vast majority of cases, namely in immunocompetent people infected at a young age, with a self-limiting disease of 1-4 weeks and a mortality rate less than 0.1%, the high number of affected people in endemic areas, and especially under compromised living conditions, for example refugee camps or serving the army, justifies extensive research for developing a safe and efficient vaccine.¹⁹ In endemic areas such as Asia, Africa or the Middle East, HEV infection is the most or second most common cause of acute hepatitis. Furthermore, HEV infection may cause fatal disease in pregnant woman, suggesting that preventing HEV infections in this group should be a high priority for a vaccine program.

Conversely, an HEV vaccine would also be valuable in developed countries, besides a temporary and local use after sporadic outbreaks. Travelers to endemic regions are at particular risk for HEV infections and might even be accountable for sporadic outbreaks after return to their countries. The recent reports of chronic HEV infections in organ transplant patients suggest that severely immunocompromised patients could benefit from an HEV vaccine.³³ Furthermore, patients with ongoing chronic liver disease of a different origin (e.g., HBV/HCV infection or alcohol abuse) would very likely benefit from a vaccination against HEV, as acute hepatitis E can cause fulminant liver failure in patients with chronic liver disease.²⁰ Since HEV has only one serotype² and natural acquisition of HEV infection leads to a proper protection against the virus,²¹ development of an HEV vaccine was generally considered a feasible task. However, it took extensive research over many years to bring the first vaccine candidate into a Phase II clinical trial.²²

HEV Vaccine Development

Different types of HEV vaccines have been developed over the past twodecades. Different prokaryotic and eukaryotic expression systems have been applied; for example bacteria, yeast,

animal, insect or plant cells, most approaches targeted the HEV capsid protein, using either full-length or part of the sequence from ORF2. These strategies resulted in recombinant protein vaccines, DNA vaccines or recombinant HEV-virus like particles (rHEV-VLPs) that were then tested in preclinical studies. Studies that have not proceeded from preclinical settings yet, including HEV DNA vaccines and also HEV vaccines derived from bacteria and plant-expression systems. The majority of them showed successful and appropriate immunization in small animal models such as mouse, rat and sheep, or even plant. However, only some of them were tested in large animal models such as monkeys. Among the vaccines based on bacterial expression systems, three out of five were tested in monkeys. The trpE-C2 vaccine showed a variable degree of protection,²³ whereas the 23-kDa and HEV239 recombinant vaccines demonstrated highly protective effects in Rhesus monkeys.²⁴ By contrast, HEV DNA vaccine approaches have largely not reached the late stages of preclinical developments. Despite the successful immunization of all DNA vaccines in small animal models, only one out of nine have been tested in monkeys at present. The pcHEVORF2 DNA vaccine showed only approximately 50% protection in *Cynomolgus* monkeys challenged with the HEV Mexican strain using intramuscular injections,²⁵ whereas application by 'Gene-Gun' technology via the shaved abdomen using the same vaccine resulted in increased protection against the Mexican strain.²⁶ Recent preclinical vaccine developments attempt to optimize not only antigenicity, but also vaccine delivery, such as plant cell-derived vaccines that may be applicable for oral or subcutaneous administration.²⁷

At present the most successful approach in HEV vaccine development was introduced by Purcell's group using the expression of rHEV protein by baculoviruses in *Spodoptera frugiperda* (Sf9) insect cells. This expression system has recently been further advanced in experimental settings by generating pseudoviruses delivering two HEV ORF2 genes via papillomavirus VLPs intramuscularly.²⁸

HEV Vaccine Preclinical Trial

The efficacy of the generated vaccines was assessed by immunization in different animal models. Some surveys only tested the generation of anti-HEV titers without challenging with wild-type strains, whereas some preclinical studies also analyzed the protective effects of the vaccine after rechallenge in primates^{21,23}. Among the different developed HEV vaccines, one recombinant vaccine, which was derived from the Sf9 insect cell expression system using baculovirus overexpression of ORF2, showed a superior protection efficiency compared with other vaccines.²⁹ This 56-kDa HEV vaccine was designed based on the HEV ORF2 of the SAR-55 Pakistani isolate (amino acid 112-607), and showed proper immunization outcome in a preclinical trial in Rhesus monkeys after challenging with different wild-type strains.³⁰ After two doses of immunization, Rhesus monkeys were protected from HEV infection by Pakistani (homologous) and Mexican (heterologous) isolates.²⁹ The administration of three doses of vaccination resulted in a longer period of protection compared with two doses of the vaccine.³⁰ In the last part of the preclinical development, immunized monkeys were challenged by three different viruses (strains Sar-55, Mex-14 and USA-2), and anti-HEV titers were then quantified against a WHO standard. The vaccine was highly efficacious in preventing HEV infection of all three wild-type strains, and titers of 175 or more WHO units protected against hepatitis and infection in monkeys. These data provided the basis to start clinical trials in humans.

HEV Vaccine in Phase I and II Clinical Trials

The HEV vaccine Phase I clinical trial was conducted by the Walter Reed Army Institute of Research using the rHEV vaccine, which was produced by DynCorp (Rockville, MD, USA). As mentioned earlier, the rHEV capsid antigen was produced in insect cells (Sf9) infected with recombinant baculovirus encoding HEV ORF2 (SAR-55 isolate from Pakistan). The Phase I clinical trial was performed in 88 healthy adults (age range: 18-50 years). Four different doses of

vaccines (1, 5, 20 and 40 μg) administered three times (day 0, month 1 and month 6) were tested in 22 individuals each.³¹ After 7 months, the HEV seropositivity rate was assessed. At a dose of 20 μg , 95% of the volunteers developed anti-HEV titers greater than 40 U/ml. However, it is important to note that the duration of possible protection from HEV infection was unclear from this study, as the seropositivity rates at month 6 (after only two doses of vaccine) ranged from 10 (1 μg) to 24% (20 μg) and 48% (40 μg).³³ Furthermore, experimental evidence whether anti-HEV serum titers greater than 40 U/ml are truly sufficient to confer protection from HEV infection in humans is lacking at present.

No serious adverse effects were reported in the Phase I trial. The most common mild adverse effect was injection-site pain. However, at the highest concentration of 40 $\mu\text{g}/\text{dose}$, one volunteer developed herpes Zoster virus and oral herpes.³¹ Owing to its greater immunogenicity and tolerability, 20 $\mu\text{g}/\text{dose}$ was chosen when conducting the Phase II clinical trial in a hyperendemic area.

A Phase II clinical trial was then conducted from 2001 to 2004 in Nepal, where HEV is highly endemic. The design of the study has been a matter of controversial ethical debates, as the study was performed on male Nepalese soldiers and paid for by the US Army and GlaxoSmithKline. Original plans for testing the vaccine on the Nepalese community were opposed by local authorities, because Nepalese residents would not have access to the vaccine after the trial ended.³² Nevertheless, 2000 healthy adults with low-to-negative anti-HEV titers underwent randomization in a double-blind, placebo-controlled trial for the rHEV vaccine, and 1794 individuals were finally treated according to the study protocol.³³ A total of 896 participants received three doses of the rHEV vaccine (20 μg rHEV antigen in 0.5 ml buffered saline plus 0.5 mg aluminium hydroxide) intramuscularly at months 0, 1 and 6, while 898 individuals received a placebo. As in the Phase I trial, administration of the vaccine was safe - the only significant side effect was injection-site pain.

The primary end point of the study was the development of acute hepatitis owing to an HEV infection, and the vaccination cohort was followed for a median of 804 days. A total of 111 episodes of acute hepatitis were evaluated by the study-monitoring board, and 87 of them could be related to HEV infection. Among these 87 patients with hepatitis E, nine had received a vaccine and 78 placebo, relating to a total vaccine efficiency of 88.5% (Figure 4). Among the subjects that completed three doses of vaccine, hepatitis E developed in 69 cases (the 66 persons from the placebo group and three from the vaccine group), relating to a rHEV efficiency of 95.5% after three doses.³³

Future Perspective

After decades of extensive research, we have now reached a situation in which one promising candidate, the rHEV 56-kDa vaccine, has been successfully and safely applied in humans and prevented hepatitis E disease at least in a short observation period of 1.5 years. However, the future direction of this vaccine program remains to be determined, as economic considerations delayed the necessary further steps of the vaccine testing. At present, it appears that an HEV vaccine may become available to travelers from the developed world and possibly to immunocompromised or organ transplant patients within the next decade, but will not be easily available to people in endemic areas that may benefit most from protection against HEV (e.g., pregnant women, refugees and adolescents). The final cost of the vaccine will be definitely an important factor in the future¹⁹

Moreover, the encouraging results from the Phase II trial should not prompt us to ignore potential obstacles related to this vaccine. The duration of protection is unclear (and appears rather limited than life long) and the efficacy in preventing the spread of HEV has to be determined. Therefore, improved formulas or different vaccines should still be investigated. Furthermore, HEV is naturally an oral-fecal pathogen; it may be more effective to design a vaccine that uses the natural way of infection (orally), thereby combining mucosal and humoral protection against HEV.³⁴

Another important field for basic and clinical research is to delineate the different routes of HEV infection and the potential (zoonotic) reservoirs. There are several reports suggesting other routes of HEV transmission than fecal-orally; for example, infection after transplantation or blood-product transfusion.⁴ Increasing reports indicate that zoonotic reservoirs such as swine, especially for HEV genotypes 3 and 4, and zoonotic transmission routes may be of exceptional relevance in immunocompromised patients⁶ These routes may require different types of preventions, and the rHEV vaccine currently tested may not sufficiently prevent disease related to HEV genotypes 3 or 4.

Conclusion

Infections with HEV are an important cause of morbidity and mortality in endemic regions, accounting for a large number of cases of acute hepatitis and representing a significant risk for pregnant women. Many different vaccine strategies have been tested, but up to now, only the rHEV 56-kDa vaccine has passed preclinical and clinical trials in primates and humans. This rHEV vaccine is produced as a recombinant peptide from the HEV capsid protein using a recombinant baculovirus and the insect Sf9 cell line. Results from clinical Phase I and II trials proved that this vaccine is safe, fairly efficient and can indeed prevent HEV in hyperendemic regions. Further studies are needed to validate appropriate indications for this vaccine and to determine its long-term effects.

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