Veterinary Microbiology 207 (2017) 1–6

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Different susceptibility and pathogenesis of rabbit genotype 3 hepatitis E virus (HEV-3) and human HEV-3 (JRC-HE3) in SPF rabbits

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ARTICLE INFO

Keywords:
HEV genotype 3 (HEV-3)
Infection
Pathogenesis
Rabbits

ABSTRACT

Hepatitis E virus (HEV) is an increasingly important zoonotic infection in humans with HEV genotypes 3 and 4 being recognized as zoonotic pathogens. The relatively recently isolated genotype 3 rabbit HEV (rHEV-3) and the more well known genotype 3 isolates from humans and swine (hsHEV-3) have all been confirmed experimentally to be capable of infecting both non-human primates and specific-pathogen free (SPF) pigs. In a previous study rHEV-3 was shown to cause acute hepatitis in experimentally infected rabbits. However, whether hsHEV-3 can productively infect rabbits remained unclear. The objective of this study was to investigate the experimental infection of rabbits with human HEV-3 (hHEV-3, JRC-HE3), to compare it to that with rHEV-3 (CHN-BJ-rb14) and to further characterise the pathogenesis of the two isolates. All animals inoculated with rHEV-3 (CHN-BJ-rb14) became infected, exhibiting an intermittent viremia, elevated liver enzymes, and persistent fecal virus shedding throughout the 15 week study period. Liver histopathology showed acute inflammation and both positive- and negative-stranded viral RNA was detected in various tissues from necropsied rabbits. By contrast, neither sero-conversion nor alanine aminotransferase (ALT) elevation was observed in most rabbits inoculated with hHEV-3 (JRC-HE3). In addition, rHEV-3 (CHN-BJ-rb14) but not hHEV-3 (JRC-HE3) recovered from primary infected rabbits was transmissible to naive rabbits. These results showed that SPF rabbits are readily susceptible to infection with rHEV-3 (CHN-BJ-rb14) but not hHEV-3 (JRC-HE3), which might indicate the influence of viral genomic organization on its pathogenicity.

1. Introduction

Hepatitis E virus (HEV) infection is the major cause of acute viral hepatitis worldwide (Kamar et al., 2012a). The virus is primarily transmitted via the fecal-oral route and in the general population causes an acute, self-limiting hepatitis with low mortality (Purcell and Emerson, 2008). However, a growing body of evidence suggests that HEV infection can also lead to chronic hepatitis particularly in immune-compromised individuals, including those that have received solid-organ transplants (SOT), those undergoing cancer chemotherapy, and HIV-infected individuals (Kamar et al., 2008; Dalton et al., 2009; Tavitian et al., 2010). Non-hepatic manifestations associated with HEV infection, such as neurologic disorders and pancreatitis have also been documented (Deniel et al., 2011; Kamar et al., 2012b; Wang et al., 2017).

HEV is a non-enveloped virus currently classified into the genus *Orthohepevirus* in the *Hepeviridae* family. It has a single-stranded positive-sense RNA genome of approximately 7.2 kb, that contains three open reading frames (ORFs) (Smith et al., 2014). At least four major human pathogenic genotypes of HEV have been recognized to-date (Smith et al., 2014). Genotypes 1 and 2 only appear to infect humans. By contrast genotypes 3 and 4 are thought to be zoonotic and appear to have a wider host range (Kamar et al., 2012a; Dalton et al., 2014; Perez-Gracia et al., 2014; Arends et al., 2014; Kamar et al., 2014; Ahmed et al., 2015). At present, swine and rabbits are recognized as the main animal reservoirs for HEV (Pavio et al., 2015; Xia et al., 2015a). The first animal genotype 3 HEV (HEV-3) strain was isolated from swine, but zoonotic HEV-3 strains have also been isolated from humans, pigs and deer worldwide (Meng et al., 1997; Tei et al., 2003; Hakze-van et al., 2011; de Paula et al., 2013). Rabbit HEV (rHEV) has been isolated in recent years from rabbits in China, USA, France and Germany (Zhao et al., 2009; Cossaboom et al., 2011; Izopet et al., 2012; Eiden et al., 2016). Phylogenetic analysis showed that these rHEV isolates are most closely related to genotype 3 and consequently they have been assigned

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http://dx.doi.org/10.1016/j.vetmic.2017.05.019
Received 20 April 2017; Received in revised form 20 May 2017; Accepted 22 May 2017

to HEV-3 (Smith et al., 2014). However overall sequence identity between rabbit HEV-3 (rHEV-3) and genotype 1–4 HEV isolates is less than 80%. In addition, all rHEV-3 isolates made to-date are characterized by an insertion of 31-aa in the X domain of ORF1, which does not exist in other genotype 1–4 HEV isolates. However, whether this insertion influences virus infectivity and pathogenicity is still unclear and warrants further investigation. In addition to the sequence similarities between rHEV-3 and HEV-3 isolates made from humans and swine (hsHEV-3), they have both been shown to be able to productively infect non-human primates and specific-pathogen free (SPF) pigs (Meng et al., 1998; Halbur et al., 2001; Cossaboom et al., 2012; Liu et al., 2013).

SPF rabbits experimentally infected with rHEV-3 developed either a typical acute hepatitis or chronic hepatitis (Han et al., 2014) and pregnant rabbits infected with rHEV-3 showed both miscarriage and high mortality (Xia et al., 2015b). However, it has remained unclear whether hsHEV-3 can replicate productively and induce hepatitis in rabbits (Cheng et al., 2012). Therefore the main objective of this study was to ascertain if human HEV-3 (hHEV-3) can productively infect rabbits and to systematically monitor the replication and pathogenesis of hHEV-3 and rHEV-3 in SPF rabbits.

2. Materials and methods

2.1. Experimental animals

Forty-five, 8-week-old, SPF New Zealand White rabbits (1–1.5 kg) were obtained from the Department of Laboratory Animal Science of Peking University Health Science Center. Pre-inoculation serum and fecal specimens were collected for 3 weeks. All animals were tested for alanine aminotransferase (ALT) to establish the baseline and were confirmed as negative for anti-HEV antibody by ELISA and were negative for HEV RNA by RT-nPCR (Han et al., 2014). Each rabbit was placed in a separate cage and fed twice a day. The animal experiments were approved by the Committee of Laboratory Animal Welfare and Ethics, Peking University Health Science Center. This study was performed in accordance with the Principles of Laboratory Animal Care (NIH publication 86-23 revised 1985).

2.2. Virus strains

The CHN-BJ-rb14 (Genbank JQ768461) strain of rHEV-3 and the JRC-HE3 (Genbank AB630971) strain of hHEV-3 were used as challenge viruses. Control HEV-negative inoculum was prepared from fecal samples of non-infected SPF rabbits. 10% (wt·vol^{-1}) suspensions [in phosphate-buffered saline (PBS) pH 7.4] of feces containing rHEV-3 or hHEV-3 were prepared as challenge stocks. The viral suspension was centrifuged for 30 min at 5000 rpm and 4 °C and then filtered sequentially through 0.45 and 0.22-μm filters before inoculation. The titers of the rHEV-3 and hHEV-3 strains in the final fecal suspensions were 2.5×10^6 and 3.0×10^6 copies·ml^{-1} respectively, as determined by the previously described TaqMan RT-qPCR (Jothikumar et al., 2006).

2.3. Infectivity titration of rHEV-3 in SPF rabbits

To investigate the pathogenicity of rHEV-3 in its natural host, the infectivity titer of the rHEV-3 stock was determined in rabbits. Briefly, ten-fold dilutions of the standard pool of rHEV-3 were prepared in PBS. Twenty-four SPF rabbits were divided at random into 6 groups (I–VI) of 4 rabbits each. The rabbits in group I–V were inoculated intravenously (i.v.) with 1 ml of a 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, or 10^{-5} dilution of the standard challenge stock, while the rabbits in group VI were inoculated intravenously (i.v.) with 1 ml of the control HEV-negative inoculum (Table 1).

2.4. Experimental infection of rabbits with rHEV-3 and hHEV-3

Fifteen SPF rabbits were divided randomly into 3 groups (A-C) of 5 rabbits each. The rabbits in group A and B were inoculated intravenously (i.v.) with 1 ml of the rHEV-3 and the hHEV-3 challenge stock respectively, while the rabbits in group C received 1 ml of the control HEV-negative inoculum (Table 2).

2.5. Passage of rHEV-3 and hHEV-3 to naïve rabbits

For further confirmation of the susceptibility of rHEV-3 and hHEV-3 in SPF rabbits, fecal suspensions positive for HEV RNA from primary inoculated rabbits were used to inoculate naïve rabbits. For this, a 10% fecal suspension positive for hHEV-3 was collected from the rabbit B4 at 1 week post inoculation (wpi), and a 10% fecal suspension positive for rHEV-3 was obtained from the rabbit A2 also at 1 wpi. 1 ml of these rHEV-3 and hHEV-3 suspensions were each used to infect 3 naïve rabbits.

2.6. Sample collection and processing

Samples of serum and feces were collected weekly after inoculation. All fecal samples were diluted in PBS to make 10% (wt·vol^{-1}) suspensions. The amount of HEV RNA in feces and serum was determined by RT-qPCR (Jothikumar et al., 2006). Serum samples were tested for ALT levels using standard methods on a Hitachi Automatic Clinical Analyzer 7180 and for anti-HEV antibody using a commercial HEV-specific sandwich ELISA according to the manufacturer's instructions (Wantai, Biopharmaceutical, Beijing, China) (Han et al., 2014). Signal-to-cutoff (S/CO) values for anti-HEV antibody were calculated and values > 1 were considered positive (Wang et al., 2002). If the serum ALT level exceeded the baseline ALT level by > 2-fold, as defined by a peak ALT value that was equal to or greater than double the pre-challenge values, a necropsy was performed. Bile and various tissues and organs including liver, kidney, duodenum, spleen, heart, bladder, and lung, were collected and stored at −80 °C. Approximately 100 mg of each tissue was homogenized in 1 ml of TRIzol reagent (Invitrogen, Burlington, ON, Canada) and clarified by centrifugation at 12,000 rpm for 15 min at 4 °C. The supernatants from this centrifugation and the bile were collected and stored at −80 °C prior to being used to detect positive/negative-stranded HEV RNA (Liu et al., 2013; Han et al., 2014). Liver and lung tissue samples were also processed for hematoxylin and eosin (HE) staining, immunohistochemistry (IHC) (microscope equipped with a digital camera, Olympus CX31, Japan) and immunofluorescence (inverted fluorescence microscope, Leica DMI3000B) by first being fixed in 10% neutral buffered formalin immediately following sampling (Han et al., 2014). To prevent cross-contamination during necropsy, individually wrapped, sterile disposable materials and new sterile scalpel blades were used for each sample.

3. Results

3.1. Infectivity titration of rHEV-3 in SPF rabbits

All rabbits inoculated with the 10^{-1} and 10^{-2} dilutions of the virus challenge stocks became infected as determined by the detection of HEV RNA in feces (Table 1). Two of four rabbits inoculated with the 10^{-3} dilution shed virus in feces and sero-converted for anti-HEV antibodies, while the other two animals in this group had no evidence of infection (Table 1). None of the rabbits inoculated with the 10^{-4} and 10^{-5} dilutions of rHEV-3 or those inoculated with the control HEV-negative inoculum were infected (Table 1). Therefore, the infectivity titer of the rHEV-3 challenge stock was 10^3 50% rabbit infectious doses (RID_{50}) per ml. Persistent fecal shedding of HEV RNA was detected from 1 wpi in the four rabbits in group I, inoculated with the highest dose of rHEV-3, and HE staining of liver tissue of animals in this group