

Review

Advances in Animal Models of Hepatitis B Virus Infection

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Abstract

Hepatitis B virus (HBV) infection seriously affects human health. Stable and reliable animal models of HBV infection bear significance in studying pathogenesis of this health condition and development of intervention measures. HBV exhibits high specificity for hosts, and chimpanzee is long used as sole animal model of HBV infection. However, use of chimpanzees is strictly constrained because of ethical reasons. Many methods were used to establish small-animal models of HBV infection. *Tupaia* is the only nonprimate animal that can be infected by HBV. Use of HBV-related duck hepatitis virus and marmot hepatitis virus infection model contributed to evaluation of mechanism of HBV replication and HBV treatment methods. In recent years, development of human–mouse chimeric model provided possibility of using common experimental animals to carry out HBV research. These models feature their own advantages and disadvantages and can be complementary in some ways. This study provides an overview of current and commonly used animal models of HBV infection.

One third of global population experiences or suffers from hepatitis B virus (HBV) infection. From this value, 350 million people suffer from chronic infection. Although HB vaccine significantly reduces incidence of HBV infection, effective treatment is still lacking for people already infected with HBV. HBV displays high specificity for hosts, and other than humans, chimpanzee is the only animal used for HBV infection studies. However, owing to source and ethical reasons, use of chimpanzee is remarkably constrained. To solve dependency on using chimpanzees in experiments, people developed a variety of alternative HBV infection models, including *Tupaia* model, which can be naturally infected with HBV, other liver-DNA virus-infected animal models closely related to HBV, and chimeric mice implanted to human hepatocytes. This study summarizes experimental animal models currently used in HBV infection studies.

Animals that can be infected by HBV, namely, chimpanzee and *Tupaia*

Chimpanzee

Chimpanzee is the first animal discovered that can be infected with HBV and widely used in evaluation of preventive HBV vaccines and drugs. Injection of serum, saliva, or semen of HBV-infected patients to chimpanzees can cause transient HBV infection [1]. Experiments showed that for genotypes A and C HBV, 10 copies of inoculated virus are sufficient to cause infection to chimpanzees [2]. In addition to being acutely infected, chimpanzees also become chronic carriers of HBV. Several years after inoculation, HBV antigen and DNA may still be detected in peripheral blood and liver puncture specimens, and chimpanzees present portal vein peripheral inflammation similar to human HB. Contrary to human HBV chronic infection, which is common in men, chronicity of chimpanzee HBV infection does not present remarkable gender differences. Although homology of chimpanzee and human genome reaches 98%, some of their immune system-related genes significantly differ. For example, the major histocompatibility class of

chimpanzees is less diverse than that of human beings. These differences lead to distinctions in HBV responses. After infection with HBV, chimpanzees rarely show symptoms and signs of hepatitis, and they also exhibit rare development of fulminant hepatitis; chimpanzees exhibit lower chronicity rate and risk of horizontal and vertical transmission of HBV infection than humans [3]. Chimpanzees are endangered species, and their relatively large physique, high experimental cost, and ethical constraints lead to their increasingly limited use in experimental research.

Tupaia

Tupaia is a small-shaped animal similar to squirrels and is closely related to primates in terms of evolution. This animal can be infected with a variety of human infectious viruses, such as herpes simplex virus, HBV, and hepatitis C virus (HCV) [4]. In China, research on HBV infection of *Tupaia* started in the 1980s, and intraperitoneal or intravenous injection of serum of HBV-infected patients can cause infection to *Tupaia* [5]. In 1996, Walter *et al.* [6] reported that after injecting *Tupaia* with serum from HBV-positive humans, HBsAg in serum lasted for two to four weeks, followed by a rapid serological conversion and production of HBsAg antibodies. Immunohistochemistry showed HBsAg and HBcAg expressions in liver tissues, and Southern blot and Northern blot revealed formation of intermediates in HBV DNA replication. HBV RNA was also synthesized in liver tissues, indicating that HBV replication, transcription, and translation occurred after the virus entered *Tupaia* hepatocytes. When serum of HBV-infected *Tupaia* was injected into another group of *Tupaia*, new HBV infection was transmitted between generations up to five times, indicating that *Tupaia* can secrete HBV infective particles after infection with HBV [7]. Kock *et al.* discovered that some components in serum affect binding of HBV with *Tupaia* hepatocytes and thus affect infection efficiency. Use of gradient centrifugation to purify HBV virus can improve efficiency of HBV infection in *Tupaia*. Previously, 108 genome equivalent virus was used to inoculate adult *Tupaia* to trigger HBV infection. This infection was maintained for two to four weeks; such duration is not conducive for studying mechanism of HBV interaction with the immune system and evaluation of antiviral drugs. Our study shows that virus inoculation

dose significantly affects during HBV infection. By adjusting dosage, we can extend HBV infection time in adult *Tupaia* to 9–15 weeks [8]. Recently, Wang *et al.* [9] observed that when newborn *Tupaia* was injected with HBV-positive serum, HBsAg and HBV DNA remained for 48 weeks in peripheral blood and liver of some *Tupaia*. This finding indicates that chronic HBV infection may occur in *Tupaia*. Yan *et al.* [10] isolated primary hepatocytes from *Tupaia*'s body, identified cell-surface receptor associated with HBV and hepatitis D virus infection, that is, sodium taurocholate cotransporting polypeptide (NTCP), and explained the mechanism of HBV infection occurring in hepatocytes of *Tupaia* at the molecular level. However, after infection with HBV, *Tupaia* presented low viral loads, and significant differences were noted among individuals. HBV infection presents low chronicity rate and lack of stability, restricting wide use of *Tupaia* in HBV research.

Animal models of HBV-associated hepadnavirus infection

For a long time, research on HBV depended on chimpanzees. However, given the high cost and ethical constraints, use of chimpanzees is limited. People explored issues related to HBV infection by studying other HBV-related hepadnaviruses.

Duck hepatitis B virus (DHBV) infection model

HBV is a type of hepadnavirus. Depending on different hosts, hepadnaviruses can be divided into avihepadnaviruses and orthohepadnaviruses. Among avihepadnaviruses, DHBV is extensively studied. DHBV exhibits high specificity for hosts, and duck carboxypeptidase D (DCPD) and glycine decarboxylase (DGD) are the major factors limiting infection specificity of DHBV [11]. DCPD can specifically bind to preS segment of DHBV large envelope protein L, but it cannot mediate DHBV infection. Furin-like protease can cut DCPD and preS segments of DHBV; thus, DHBV can enter cells that it cannot infect prior to cutting [11]. Esfahani *et al.* [12] noted that by comparing immune responses of newborn and adult ducks infected with DHBV, chronic DHBV infection mainly results from dysfunction of inherent immune system in the early phase of infection rather than flawed

responses of cellular immunity, as traditionally conceived. After infecting ducks, DHBV genome manifests mainly in the form of cccDNA, which is highly stable in cells and is insusceptible to antiviral drugs; thus, DHBV can maintain relative stability of cccDNA content in the nucleus without requiring synthesis of new DNAs^[13]. Duck sources are stable, raising and management of these animals are convenient, and cost is relatively low. Therefore, ducks are widely used in hepadnavirus research and particularly play a positive role in drug evaluation^[14]. Primary deficiencies of DHBV infection duck model include differences between evolution of ducks and humans and between structures of DHBV and HBV.

Woodchuck hepatitis virus (WHV) infection model

Mammals that can be infected by positive hepadnaviruses include primates, such as gibbons, orangutans, chimpanzees, gorillas, and woolly monkeys, and rodents, such as woodchucks, ground squirrels, and arctic squirrels^[1]. WHV is used extensively in these animals. After infecting woodchuck, WHV can be expressed as acute or chronic infection, which is related to age of woodchuck and WHV genotype^[15]. Further studies showed that chronic WHV infection of woodchuck is related to the following factors: flawed response of type I interference, intrahepatic-induced T cell depletion, increased inhibitory cytokines, such as SOCS3, and intrahepatic neutrophil aggregation^[16]. Fletcher *et al.*^[17] compared transcriptomes of woodchucks that feature self-limited WHV infection and chronic WHV infection and observed that perforin, other cytotoxic T cell activation genes, and interferon-stimulating genes play important roles in controlling WHV infection. Woodchuck WHV infection model is widely used in anti-HBV drug evaluation because immune responses and clinical manifestations of woodchucks infected with WHV are similar to those of humans similarly infected with HBV^[18]. The main drawback of WHV model includes significant differences in structures of WHV and HBV. Thus, this model cannot completely simulate HBV infection in humans.

HBV-infected human/*Tupaia*-mouse chimeric liver model

Mice are the most commonly used experimental animals.

However, mice cannot be infected with HBV. Human-mouse chimera, which is formed by transplanting human hepatocyte in mice, can be used to solve this problem. Human-mouse chimeric liver model displays the following commonalities: 1) death of original hepatocytes induced by transgenes provides growth space and microenvironment for exogenous-transplanted hepatocytes; 2) host adaptive immune system defects allow allogeneic-transplanted cells to survive in the body^[19].

The first established chimeric model was a trimera mouse. Hematopoietic cells of Balb/c mice were removed after irradiation with lethal doses. The hematopoietic system was reconstructed with bone marrow of severe combined immunodeficient (SCID) mice. Then, HBV-infected liver tissues were transplanted to renal capsules of mice. Up to 85% of mice presented HBV viremia^[20]. Human liver tissues with such model transplant cannot maintain normal tissue structure. Human hepatocytes possess short survival time, and viral load in mouse body is low. Transplant of normal human hepatocytes to mice cannot cause HBV infection. Therefore, this method is not applicable for detecting early mechanism of HBV infection. Ohashi *et al.*^[21] transplanted normal human primary hepatocytes to renal capsules of non-obese diabetic (NOD)/SCID immune-deficient mice and injected excitatory antibody of hepatocyte growth factor receptor c-Met, which can significantly prolong survival time of human hepatocytes in mice. HBsAg and HBV DNA in serum of HBV-infected mice can last for five months. The main drawback of this mouse model is significantly limited proliferation of exogenous hepatocytes in mouse body.

Another kind of mice used included urokinase-type plasminogen activator (uPA) transgenic mice, which were injected with uPA gene. Under the effect of albumin promoter and enhancer, high expression of uPA gene in mouse liver resulted in death of hepatocytes in newborn mice, creating growth space for foreign hepatocytes^[19]. In progeny of uPA transgenic mice and recombination activating gene (RAG)-2^{-/-} mice, uPA/RAG-2^{-/-} mice lacked mature T lymphocytes and B lymphocytes. Transplanted human hepatocytes can multiply in mouse liver and occupy 15% of liver parenchymal cells; thus, stable infection can be established after HBV inoculation^[22]. The *Tupaia*-mouse chimera formed by transplanting *Tupaia* primary hepatocytes to uPA/RAG-2

$^{-/-}$ mice can also be infected with HBV and respond to antiviral drugs [23]. uPA/SCID mice can be obtained by hybridization of uPA transgenic mice with SCID mice, and transplanted exogenous hepatocytes can survive in the liver for long periods and proliferate [24]. Lutgehetmann *et al.* [25] used HBV-infected uPA/SCID human–mouse chimeric model, and results showed that HBV inhibited STAT1 from translocating to the nucleus and blocked signal transduction pathway and antagonized antiviral activity of IFN- α . This phenomenon benefits establishment and maintenance of chronic HBV infection. One of the major defects of uPA mice is high mortality of new homozygous uPA transgenic mice. This condition hinders establishment of breeding system of pure uPA transgenic mice.

To overcome shortcomings of low reproducibility, high mortality rate of newborn mice, and short duration of transplant time of uPA transgenic mice, Azuma *et al.* [26] hybridized fumarylacetoacetate hydrolase (Fah) $^{-/-}$ mice with RAG-2 $^{-/-}$ /Il2rg $^{-/-}$ mice and obtained Fah $^{-/-}$ /RAG-2 $^{-/-}$ /Il2rg $^{-/-}$ mice. Fah $^{-/-}$ mice lacked Fah, and the disorder occurred during end stage of tyrosine catabolism, leading to accumulation of tyrosine intermediate metabolites and resulting in liver injury. 2-(2-Nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) can inhibit activity of 4-hydroxyphenyl pyruvate dioxygenase, reduce generation of downstream metabolites, and prevent occurrence of liver injury. When NTBC was added to food, Fah $^{-/-}$ mice survived for a long time. When NTBC was removed, Fah $^{-/-}$ mice experienced hepatocytic injuries, allowing long-term survival and proliferation of transplanted human hepatocytes. The recently established Fah $^{-/-}$ /NOD/SCID mice also allowed for transplantation and expansion of human hepatocytes [27]. These two kinds of mice were easily used to achieve mass growth and proliferation of primary hepatocytes. Thus, these animals can be easily used for breeding and manual control. uPA transgenic mice display several advantages in HBV infection research.

Summary and prospect

HBV infection animal model is an indispensable tool for studying mechanisms of HBV infection, pathogenesis, interaction with the immune system, and evaluation of

therapies. Currently, chimpanzees are still the most ideal animal for HBV infection model. However, limited sources and ethical factors constrain wide use of this organism. Aside from chimpanzee, *Tupaia* is the only animal that can be infected with HBV. However, *Tupaia* infection is mostly transient, which prevents long-term observation and drug evaluation of HBV infection. Recently, newborn *Tupaia* were used to establish chronic HBV infection, but low chronicity rate and large differences existed among considered individuals. As alternatives to HBV infection model, WHV and DHBV infection models play important roles in explaining virus–host interaction mechanism and evaluating treatment of hepadnaviruses. However, as a result of genetic background differences, conclusions obtained from these alternative models cannot fully reflect the state of HBV in the human body. Human–mouse chimera involves transplanting human liver tissue or liver cells to mice. This model can be infected by HBV and shows complete life cycle of HBV. However, breeding uPA transgenic mice poses difficulty. Chimeric mice show high mortality, and sources of human primary hepatocytes are limited. Thus, difficulty arises from promoting this chimera in most laboratories. Fah $^{-/-}$ mice are easy to reproduce and feature high survival rate, and their hybrids with RAG-2 $^{-/-}$ /Il2rg $^{-/-}$ and NOD/SCID mice allow for proliferation and long-term survival of transplanted human hepatocytes. These hybrids can also be used as suitable models for HBV infection.

Recently, Su *et al.* [28] transplanted human hematopoietic stem cells and human liver progenitor cells to immune-deficient mice and established humanized mice with human immune system and human hepatocytes. These mice can be infected with HCV, and liver injury can occur and stimulate human immune system response. Humanized mice are improved to study HBV infection and to obtain optimal results.

Although HBV can only infect several cells, including human primary hepatocytes, *Tupaia* primary hepatocytes, and HepRG cells, HBV can replicate in various transfected cells or transgenic animal liver cells. This phenomenon shows that restrictive step of HBV infection is virus entry to cells. Discovery of HBV receptor, NTCP, will facilitate study of transgenic mice and provide a convenient animal model for HBV research.

Declarations

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No.

Competing interests

The author declares that he has no competing interest.

Authors' contributions

H Zhang made the literature analysis and wrote, discussed and revised the manuscript of this review.

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