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Review

High-pressure Hydrodynamic Injection as a Method of Establishing Hepatitis B Virus Infection in Mice

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Among several existing mouse models for hepatitis B virus (HBV) infection, the high-pressure hydrodynamic injection (HDI) method is frequently used in HBV research due to its economic advantages and ease of implementation. The use of the HDI method is influenced by factors such as mouse genetic background, age, sex, and the type of HBV plasmid used. This overview provides a multidimensional analysis and comparison of various factors that influence the effectiveness of the HBV mouse model established through HDI. The goal is to provide a summary of information for researchers who create HBV models in mice.

Abbreviations and Acronyms: cccDNA, covalently closed circular DNA; HBV, hepatitis B virus; HDI, hydrodynamic injection; pBS, pBluescript II KS (+)

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Introduction

Hepatitis B virus (HBV) belongs to the family Hepadnaviridae, a group of DNA viruses with strong species specificity. HBV primarily infects humans and a few primate species and is a major causative factor of liver cirrhosis and hepatocellular carcinoma. In 2019, 296 million people globally were hepatitis B surface antigen (HBsAg) positive, the prevalence of chronic HBV infection was about 3% to 5%, and HBV caused about 820,000 deaths.[13] Despite a large patient population, existing treatment methods have yet to achieve satisfactory viral clearance, and due to the narrow host range of HBV, few susceptible animal models are available for studying the virus. Currently, HBV animal models mainly include nonhuman primates, such as chimpanzees[41] and tree shrews.[15] However, the limited availability of these animals and high-costs of using them hinder the use of either of them in HBV studies.[9] Other HBV-related viruses, such as woodchuck hepatitis virus[33] and duck HBV[9,14] models, have different infection mechanisms and replication processes as compared with human HBV.

Mice are commonly used experimental animals in medical research. Although they cannot be naturally infected with HBV, their accessibility and well-defined genetic backgrounds have led many researchers to establish various mouse models of HBV infection using different methods. These models include high-pressure hydrodynamic injection (HDI), HBV transgenic models, adeno-associated virus HBV (AAV-HBV) transduction models, covalently closed circular DNA (cccDNA) replacement models, and humanized liver models,[10] all of which have provided essential tools for HBV research. Among these, HDI is frequently used in HBV research due to its efficiency and relative ease of implementation.

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Principle and Implementation of the HDI Method

HDI is used to inject a large volume of solution containing plasmid DNA (approximately 8% to 10% of the mouse body weight)[23] into the mouse tail vein over a period of 5 to 8 s. This injection volume, which is close to the mouse's circulatory volume, causes a rapid increase in blood pressure. Due to the large injection volume, the heart cannot quickly recirculate plasmid DNA after it passes through the inferior vena cava. Instead, blood flows back into the liver through the portal vein, leading to a rapid increase in liver volume and increased permeability of the capillary endothelium. This enlarges pores in cell membranes, thereby allowing the plasmid DNA to enter the cells. When the pores close, the plasmid DNA remains in the liver cells.[36] This process prevents plasmid DNA from being degraded by DNA enzymes in the bloodstream and enhances transfection efficiency. In contrast, after a typical tail vein injection, plasmids enter the bloodstream more slowly and are then distributed by the circulatory system at a normal pressure; the liver remains at the same pressure as the rest of the major arterial vessels, and normal flow-through occurs. However, due to the presence of numerous nucleases in the blood and other organs, including on tissue, plasmids are rapidly degraded in the circulatory system or recognized and absorbed by nonparenchymal liver cells.[17]

The HDI method was first used in 1999 to inject plasmid DNA solution into the mouse tail vein, showing that this approach could achieve high-level expression of foreign genes.[47] In 2002, HDI was used HDI to inject plasmids containing the HBV genome, thereby creating an acutely HBV-infected mouse.[45]

Since then, a number of research teams have conducted in-depth research by using this simple and convenient in vivo transfection method.[16]

Factors that Influence the HDI HBV Model

The expression level and persistence of HBV after HDI are highly dependent on factors such as plasmids and vector frameworks and host genetic background, age, and sex. These factors must be considered in the implementation of HDI method of HBV infection.

Plasmid. The plasmid of HBV is perhaps the crucial factor determining the expression level and persistence of HBV in mice. Common plasmids include pAAV,[11] pBluescript II KS(+) (pBS),[19,20] pcDNA3.1(+), and[27] pSM2.[28,43] Among these vectors, pAAV is probably the most commonly used. AAV is a single-stranded linear DNA parvovirus that promotes high infection efficiency, low pathogenicity, low immunogenicity, and the ability to express foreign genes for extended periods. Delivery of HBV genes via pAAV can lead to long-term expression of HBsAg and a tolerant phenotype with the minimal liver inflammation.[44] pBS is derived from the pUC19 plasmid vector and is a nonviral vector with multiple cloning sites; it is commonly used for cloning and sequencing. pcDNA3.1(+) is a versatile nonviral vector used for both high-level stable and transient expression in mammalian hosts. pSM2 is a lentiviral/retroviral vector with high transfection efficiency (Table 1). pAAV and pcDNA3.1(+) carrying the HBV genome can achieve high and sustained expression in mice, making them suitable for establishing HBV infection models.

Dose. The persistence of HBV in mice is notably influenced by the dose of the administered plasmid. A study of varying doses of the pAAV-HBV1.2 plasmid in mice (a 1.2-fold HBV genome)[2] revealed that a plasmid dose of 6 µg induces immune tolerance, thereby sustaining continuous HBV infection. In contrast, 20 µg of plasmid induced antigen recognition receptors and triggered an anti-HBV immune response that cleared HBV in a short period of time.[40] Consequently, implementation of the HBV HDI model requires careful consideration of both the plasmid structure and the injected dose. Using high-dose HBV plasmids tends to expedite the clearance of HBV in mice. Therefore, using a large dose of HBV DNA plasmid is generally not advisable for establishing a chronic HBV infection model.

Genetic background. The genetic background of mice plays a significant role in their HBV phenotypes. Currently, numerous mouse strains can be used; the appropriate strain should be selected based on the research objectives. Acute HBV infection using HDI in C57BL/6 mice is the classic HBV model. In 2006, one group established an HDI model using pAAV-HBV in C57BL/6 mice; 40% of the mice exhibited sustained HBsAg expression for over 6 mo, simulating long-term HBV expression and providing new approaches to studying chronic HBV

Table 1. Effects of different plasmid on HBV HDI model

Plasmid	Dose	Mouse strain	Sex/age	HBsAg expression level and persistence	Result	Reference
pcDNA3.1(+)-HBV1.3C	15 µg	C57BL/6	Male/6–8 wk	OD450 of HBsAg near 3.5 at week 1, maintained around 2.5 during 2–5 wk, and 1.5 from 6 to 20 wk	pAAV-HBV1.2A showed better persistence than pcDNA3.1(+)-HBV1.3C and pAAV-HBV1.3C	22
pAAV-HBV1.3$_C$				Rapid decrease in OD450 of HBsAg to below 0.5 by week 1, cleared by 3 wk		
pAAV-HBV1.2$_A$				OD450 of HBsAg at 3.0–3.5 for the first 2 wk, followed by a decline, completely cleared by 12 wk		
pCS-HBV1.3	5 µg	C57BL/6	Female/6 wk	HBsAg maintained at 10^2–10^3 IU/mL level for over 8 wk	pAAV-HBV1.3 had higher HBsAg levels in 2–3 wk than pCS-HBV1.3, but pCS-HBV1.3 had better persistence	5
pAAV-HBV1.3				HBsAg levels higher than pCS-HBV1.3 in 2–3 wk, then rapidly decreased and completely cleared by 4 wk		
pBS-HBV1.1$_B$	10 µg	BALB/c	Male/6 wk	HBV DNA, 2–4 l-g copies/mL (0–7 dpi)	pBS-HBV1.3$_B$ had higher HBV DNA expression than pBS-HBV1.1$_B$ and pBS-HBV1.2$_B$	19
pBS-HBV1.2$_B$				HBV DNA, 4–6 l-g copies/mL (0–7 dpi)		
pBS-HBV1.3$_B$				HBV DNA, 6–8 l-g copies/mL (0–7 dpi)		
BPS	10 µg	C57BL/6	Male/6–8 wk	OD450 of HBsAg maintained around 3 for the first 3 wk, then completely cleared after 3 wk	BPS showed better persistence than pSM2	43
pSM2				OD450 of HBsAg maintained at 3–4 for the first week, then rapidly decreased below BPS group, completely cleared after 3 wk		

dpi, days postinfection.

Table 2. Effects of different mouse genetic backgrounds on the HBV HDI model

Mouse strain	Sex/age	Plasmid	Dose	HBsAg expression level and persistence	Result	Reference
C57BL/6J	Male/6 wk	pAAV-HBV1.2	10 μg	HBsAg positivity rate 40% at 8 wk, 15% after 6 mo	CBA/CaJ had the longest persistence, highest positivity rate	4
C3H/HeN				HBsAg positivity rate 90% at 20 wk, 80% after 6 mo		
DBA/2J				HBsAg positivity rate 70% at 8 wk, 25% after 6 mo		
CBA/CaJ				HBsAg positivity rate 100% at 21 wk, 80% after 6 mo		
BALB/cJ				Rapid clearance of serum HBsAg within the first 4 wk		
FVB/NJ				Rapid clearance of serum HBsAg within the first 4 wk		
NOD/ShiLtJ				Rapid clearance of serum HBsAg within the first 2 wk		
129 × 1/SvJ				Rapid clearance of serum HBsAg within the first 2 wk		
C57BL/6J	Male/6 wk	pAAV-HBV1.2	10 μg	HBsAg at 10^3–10^4 ng/mL within the first 8 wk, nearly 40% of mice still detectable at 26 wk	C57BL/6J had higher HBV DNA and longer persistence than C57BL/6N	38
C57BL/6N				HBsAg positive within the first 8 wk, rapidly dropped below detection limit after 8 wk		

infection.[11] Another group found that C3H/HeN mice showed higher and more persistent HBV expression than C57BL/6 mice after HDI. Differences in HBV infection related to genetic background have been described in different mouse strains and strains (Table 2).[30] C57BL/6 substrains have significant phenotypic differences in physiology, biochemistry, and immunocompetence, which result in differential susceptibility to virus-induced inflammatory disease.[29] Viral clearance differs among different C57BL/6 substrains created using HBV HDI. Around 40% of C57BL/6J mice had detectable HBsAg and HBV DNA in their serum at week 26, while HBsAg and HBV DNA dropped below detection limits by week 8 in C57BL/6N mice.[38] The use of HDI to establish the HBV infection in transgenic mice via the human AAVS1 site element transgenic mice resulted in high-level and sustained expression of HBV genes.[46] C57BL/6J, CBA/CaJ, and C3H/He mice exhibit higher and more persistent HBV expression as compared with NOD/ShiLtJ mice and are therefore better choices for establishing HBV mouse models.

Age. Another crucial factor that affects HBV clearance is age. In adult humans, some proportion of infected individuals can clear the virus from their bodies, whereas newborns and children tend to have high rates of virus replication after HBV infection,[8,25,34] often leading to chronic infection. Juvenile mice (approximately 6 to 8 wk)[8] show age-dependent HBV clearance (Table 3), partly because HBV can overcome the immature liver immune functions to establish a chronic infection. As mice age, their immune systems gradually mature and become more efficient at HBV clearance. Adult mice (approximately 8 to 12 wk)[7] have a mature liver immune system and exhibit strong immune responses to HBV.[6,12,42]

Gut microbiota. The ability of mice to clear intrahepatic HBV is closely related to their gut microbiota. As compared with juvenile mice, the gut microbiota in adult mice is relatively abundant and complex and can stimulate liver immunity and facilitate rapid HBV clearance. The addition of combinations of antibiotics to the drinking water of 6- to 8-wk-old C57BL/6 mice with established HBV HDI resulted in lower HBV clearance by mice in the antibiotic group as compared with the control group; this difference occurs mainly because antibiotics disrupt the composition and function of gut microbiota, leading to elevated levels of serum lipopolysaccharide, which in turn induce endotoxemia and inflammatory factor production, which subsequently accelerates HBV clearance.[51] Mice treated with antibiotics and subsequent fecal microbiota transplantation

Table 3. Effects of age on HBV HDI model

Age	Mouse strain	Plasmid	Dose	HBsAg expression level and persistence	Result	Reference
Young	C57BL/6	pAAV-HBV	10 μg	Around 50% of mice HBsAg positive for more than 8 wk	Young mice have higher HBsAg positivity rate than adults	12
Adult				HBsAg positivity rate drastically drops by 3 wk, completely cleared by 6 wk		
Young	DBA/2J	pAAV-HBV1.2	10 μg	HBsAg positive for more than 25 wk		4
Adult				HBsAg completely cleared by 4 wk		
Young	C3H/HeN	pAAV-HBV1.2	10 μg	HBsAg positivity rate 50% by 25 wk		4
Adult				HBsAg completely cleared by 4 wk		

Young: 6–8 wk; adult: 8–12 wk.

Table 4. Sex effects on HBV HDI model

Sex	Mouse strain	Age	Plasmid	Dose	HBsAg expression level and persistence	Result	Reference
Female	C57BL/6	6–8 wk	AAV8-1.3HBV	5×10^{10} GC	< 5,000 IU/mL (within 6 wk)	Male HBsAg higher than female	39
Male					5,000 < HBsAg < 20,000 IU/mL (within 6 wk)		
Female	B6-Tg(AAVS1)A1Xob/J	6–8 wk	pAAV-HBV	10 µg	HBsAg 10^2–10^3 IU/mL		46
Male					HBsAg 10^3–10^4 IU/mL		

were less susceptible to HBV infection.[37] This type of evidence highlights the crucial role of gut microbiota in the ability of mice to clear HBV. Therefore, the use of juvenile mice for about 6 to 8 wk is recommended when establishing the HBV HDI model.

Sex. Susceptibility to HBV infection is also related to sex. Sex hormone receptor signaling drives sexual dimorphism in gene expression throughout liver development. This sexual dimorphism is evident in the different infection rates of male and female mice after inoculation with HBV.[50] Human men tend to have a higher rate of chronic HBV infection and higher viral loads than do women. Women have stronger innate and adaptive immune responses than men. Sex hormones such as testosterone and estrogen influence systemic immune responses through hormone receptors expressed in immune cell lineages. Hormones also contribute to hepatic epigenetics and genetic alternations.[24] Mice show sex differences in HBV-related immune responses, particularly with regard to immune tolerance and clearance. Testosterone can enhance the synthesis of HBV mRNA,[35] resulting in higher HBV gene expression and viral replication levels in males as compared with females (Table 4).[39] However, the mechanisms underlying the sex hormone-regulated HBV-specific immune response remain unclear.

Advantages, Disadvantages, and Existing Challenges for HDI-HBV Models

Advantages. The HDI method is widely applicable for the study of HBV, including areas such as recombinant covalently closed circular DNA (rcccDNA) persistence and the development of new intervention strategies. Except for its wide range of applications, HDI is also highly efficient. A study that used site-specific DNA recombination technology and HDI to establish HBV infection in mice showed that HBsAg and rcccDNA persisted for more than 62 wk after inoculation, providing the opportunity to study many aspects of HBV pathobiology.[18]

In addition to HBV, the HDI method has been used to create various other disease models, including nonalcoholic fatty liver disease,[49] hemophilia B,[26] and enterovirus 71 infection.[31] Furthermore, the HDI transfection method, originally used in mice, has also been used in rats[48] and pigs.[3]

Another advantage of the HDI model is its utility for comparing different HBV genotypes and virus strains in mice, enabling rapid in vivo analysis and the basis of persistent HBV expression and potential pathogenic mechanisms.[32]

Limitations and challenges. Constructing the HDI model involves injecting a substantial volume of liquid into the mouse's tail vein, which can potentially cause short-term liver damage,[1] Hepatocyte swelling, formation of hemorrhagic necrotic foci, and elevation of alanine aminotransferase have been observed. Hepatic injury is most severe 30 min after injection and is then repaired naturally by cell proliferation in about 1 wk.[21] However, these effects could complicate the interpretation of experimental results.

The process of HDI may be painful for mice, so anesthesia or analgesia may be appropriate before HDI. The effect of anesthetics or analgesics on the efficiency of transforming rcDNA into cccDNA in mouse liver cells has not been studied. The efficiency of transformation in mouse liver cells is normally low, and anesthetics or analgesics could reduce the transfer of cccDNA into the cells, perhaps limiting the study of some HBV biologic processes.

Mice are not natural hosts for HBV, and their infection rate is considerably lower than that of human HBV patients. Consequently, the HDI model cannot fully replicate the entire course of human HBV infection and has limitations as a model of natural infection.

Summary

The establishment of a useful animal model of HBV infection requires high-level and sustained HBV gene expression and replication. Among various transfection models, the HDI model is widely used. However, this model faces some challenges, including liver damage during injection, low cccDNA formation efficiency, and the nonnatural host status. In addition, the HDI method is stressful for mice, and studies have not determined whether the use of anesthetics or analgesics will provide similar successful outcomes. However, using mice allows the replacement of primates with a less sentient species for at least some types of studies and is much less costly. Institutional ethics committees or IACUCs should review this model according to their respective regulatory standards, with due consideration of the health of hundreds of millions of people who are infected with HBV. Addressing the challenges of using HDI to create HBV infections in mice is critical to advance HBV research. New techniques to improve transfection efficiency and minimize pain and trauma in animals will be particularly valuable in the future as we strive to increase our understanding of human HBV infection.

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Conflicts of Interest

The authors declare no conflict of interest.

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