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Nucleoside analogues alone or combined with vaccination prevent hepadnavirus viremia and induce protective immunity: Alternative strategy for hepatitis B virus post-exposure prophylaxis

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ABSTRACT

Objectives: The current strategies for hepatitis B virus (HBV) post-exposure prophylaxis (PEP) are not generally available in remote and rural areas of developing countries and/or carry potential risks for infection with blood-borne transmitted pathogens. Nucleotide analogues (NAs) are successfully used for human immunodeficiency virus PEP, and maybe effective for HBV PEP. In this study, we tested the NA-based strategies for HBV PEP using the Chinese woodchuck model.

Methods: Chinese woodchucks were inoculated intravenously with different doses of woodchuck hepatitis virus (WHV). A deoxyguanosine analogue entacavir (ETV), a DNA vaccine pWHclm, or ETV plus pWHclm were applied to the infected animals 24 h later. Twenty weeks later, the animals were re-challenged with WHV to test for the presence of immunity against WHV.

Results: Inoculation with different WHV doses had a strong influence on the course of WHV infection; NA alone or in combination with a DNA vaccine completely prevented viremia after a high dose of WHV inoculation in Chinese woodchucks and induced partial or complete protective immunity, respectively.

Conclusions: NA-based PEP strategies (NA alone or in combination with vaccine) may be an alternative of HBV PEP, especially in those living in the remote and rural areas of the developing countries and the non-responders to the current vaccine, and may be valuable in the PEP of HBV and HIV co-infection after occupational and non-occupational exposure. Further clinical studies are warranted to confirm the valuable of NA-based strategies in HBV PEP.

1. Introduction

Hepatitis B virus (HBV) infection is widely distributed throughout the world. An estimated 2 billion people have been infected with HBV worldwide. At least 350 million people are HBV carriers and are at high risk for developing hepatic decompensation, cirrhosis, and hepatocellular carcinoma (Liaw and Chu, 2009). The prevalence of HBV infection in China was 7.18%, and there are approximately 93 million patients with chronic HBV infection in China (Liang et al., 2009; Lu and Zhuang, 2009). Thus, HBV infection remains a serious public health issue in China and the rest of the world.

Health care personnel (HCP) are at high risk of HBV infection because of accidental exposure to blood from HBV patients or injury from needles contaminated with HBV patients' body fluids

Abbreviations: HBV, hepatitis B virus; HCP, health care personnel; PEP, post-exposure prophylaxis; NA, nucleoside (nucleotide) analogue; HIV, human immunodeficiency virus; ART, antiretroviral therapy; WHV, woodchuck hepatitis virus; WHcAg, WHV core antigen; WHcAb, WHV core antibody; WHsAg, WHV surface antigen; WHsAb, WHV surface antibody; ETV, entacavir; GE, genome equivalent; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; LAM, lamivudine.

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(Werner and Grady, 1982). Currently, the strategies for post-exposure prophylaxis (PEP) for HBV infection includes observation, hepatitis B vaccine immunization, or hepatitis B vaccine immunization combined with high titers of hepatitis B immunoglobulin, depending on the status of HBV infection, previous hepatitis B vaccine immunization, and the antibody responses of the exposed persons (U.S. Public Health Service, 2001). However, the commonly used hepatitis B immunoglobulin is a blood product, which carries the potential risks of infection with blood-borne transmitted pathogens. Moreover, the transportation and storage of hepatitis B vaccine and hepatitis B immunoglobulin might be limited in certain remote and rural areas of developing countries. Recent studies indicated that the coverage rate of routine hepatitis B vaccination in HCPs is poor in developing country (Ziglam et al., 2013), and partially caused by the related travel costs and the time because of the inconvenient traffic, especially in the remote and rural areas (Zhu et al., 2013). It is therefore necessary to develop new strategies for HBV PEP.

Nucleoside (nucleotide) analogues (NAs) demonstrate definite inhibitory effects on HBV and human immunodeficiency virus (HIV) replication, and they are widely used in the treatment of chronic hepatitis B patients and patients with HIV infection. Following accidental exposure, HCPs are also at risk of HIV infection as well as HBV infection. Both animal and clinical studies indicated that NAs have optimal post-exposure prophylactic effects on accidental HIV infection (Cardo et al., 1997; Tsai et al., 1995); therefore NAs are currently recommended in the PEP for HIV infection (Panlilio et al., 2005; Smith et al., 2005). NAs treatment alone or combined with vaccination prevented duck HBV persistence in newborn ducks (Feng et al., 2010; Foster et al., 2005; Miller et al., 2008). Two recent retrospective study indicates that anti-HBV NAs containing antiretroviral therapy (ART) reduced incident HBV infections in HIV infected patients (Gatanaga et al., 2013; Sheng et al., 2013). These findings suggested that NAs may be used for HBV PEP.

Eastern woodchuck (*Marmota monax*) infection with woodchuck hepatitis virus (WHV) strongly resembles human HBV infections in its major virological, pathological and immunological features (Summers et al., 1978). As a result, the WHV-infected woodchuck model is widely used to study HBV pathogenesis and evaluate antiviral drugs or therapeutic vaccines (Lu and Roggendorf, 2001; Menne and Cote, 2007; Roggendorf et al., 2010). The Chinese woodchuck (*Marmota himalayana*) is phylogenetically closely related to the Eastern woodchuck (Fan et al., 2012; Lu et al., 2008; Steppan et al., 1999) and is susceptible to WHV infection (Wang et al., 2011). Thus, the Chinese woodchuck model is also suitable for studies on the pathogenesis of HBV infection and for testing various prophylactic and therapeutic approaches to HBV infection. In this study, we used the newly established Chinese woodchuck model to explore NAs alone or in combination with vaccine for HBV PEP. Because the commercial hepatitis B vaccine is not suitable for the woodchuck system, WHV surface antigen is not easily available, and cellular responses are essential for the control of HBV infection, a WHV core antigen (WHcAg) expression plasmid, pWHcIm, which can induce potent cellular responses against WHcAg, was used in this study. Our results demonstrate that NAs in combination with specific HBV vaccines may be an alternative strategy for HBV PEP.

2. Materials and methods

2.1. Animals

Chinese woodchucks were captured in the wild and provided by the Qinghai Institute for Endemic Disease Control and Prevention (Xining, Qinghai, China). Tests for WHV core antibody (WHcAb), surface antigen (WHsAg) and antibody (WHsAb), and WHV DNA were conducted to exclude previous exposure to WHV. The general information of the Chinese woodchucks used in this study is shown in Supplemental Table 1. All of the animal experiments were performed under the guidance of the Animal Ethics Committee of Tongji Medical College, Huazhong University of Science & Technology.

2.2. Drugs and plasmids

Entacavir (ETV) powder was provided by the Jiangsu ChiaTai-Tianqing Pharmaceutical Co., Ltd. (Nanjing, Jiangsu, China). ETV was added to banana at a dose of 0.5 mg kg^{-1} per day for feeding. Cardiotoxin (Sigma, St. Louis, MO, USA) was prepared as a 10 μM stock solution and was stored as 1 ml aliquots. The DNA vaccine pWHcIm was described previously, which induced potent antibody and cellular responses against WHcAg and protected woodchucks from WHV infection (Lu et al., 1999). A large-scale plasmid preparation was made using the EndoFree plasmid Giga kit (Qiagen, Hilden, Germany) and was stored as 1 ml aliquots.

2.3. Animal experimental design

To determine the course of WHV infection at a variety of infectious doses, 9 Chinese woodchucks were divided into 3 groups ($n = 3$) and infected with 10^4, 10^6 or 10^8 genome equivalents (GEs) of WHV7. Two animals received saline and served as mock controls. Blood samples were collected at the indicated time points. Serum WHV DNA and WHcAb were measured as described below.

In the second experiment, 24 Chinese woodchucks were infected with WHV and then divided into 4 groups: infection control (group A), pWHcIm vaccination (group B), ETV treatment (group C), and ETV treatment plus pWHcIm vaccination (group D). For the primary WHV inoculation, the animals were intravenously injected with a WHV7 stock at a dose of 10^8 GEs. ETV was administered 24 h later and continued for 8 weeks. After pretreatment with cardiotoxin, the Chinese woodchucks were immunized with pWHcIm 3 times at 3 week intervals, according to the procedures described in our previous work (Lu et al., 1999). A part of the Chinese woodchucks were re-challenged at week 20 with a WHV stock at a dose of 10^9 GEs. Two additional naïve animals were included as re-challenge controls (group E), receiving the WHV stock only. Blood samples were collected at the indicated time points, and the serological markers of WHV infection including WHV DNA, WHsAg, WHcAb, and WHsAb were measured as described below. The description of animal groups is shown in Supplemental Table 2.

2.4. Tests for the serological markers of WHV infection

The serum samples were diluted 1:10 in phosphate buffered saline, and the presence of WHcAb, WHsAg and WHsAb were determined by enzyme-linked immunosorbent assay (ELISA) as described previously (Lu et al., 2005; Wang et al., 2011). The results for WHsAg and WHsAb are presented as S/N values = OD of sample/OD of negative control. The results for WHcAb are presented as the percentage of inhibition = [((OD of negative control − OD of sample)/OD of negative control) × 100]. Viral DNA was isolated from serum using the E.Z.N.A® Viral DNA Kit (Omega, Doraville, GA, USA). Real-time polymerase chain reaction (PCR) was performed with a SYBR® Green Real-time PCR Master Mix (Toyobo, Osaka, Japan), according to the manufacturer's instructions. The PCR primers used for amplification were QP1 and QP2, as described previously (Wang et al., 2011). Plasmids containing the WHV full-length gene were serial diluted and used

as the standards. The detection limit of this assay was at 10^2 WHV GE per reaction.

3. Results

3.1. WHV inoculation dose influenced the course of WHV infection in Chinese woodchucks

Needle stick injury may introduce up to 0.7 μl of HBV contaminated blood containing up to 10^{5-6} HBV GEs into the exposed individual (Bennett and Howard, 1994). In chimpanzees, the size of HBV inoculums strongly influences the course and the outcome of HBV infection (Asabe et al., 2009). Therefore, we examined the influence of WHV inoculation doses on the course of infection in Chinese woodchucks to ensure the optimal dose of WHV inoculums in the following experiments.

The animals were inoculated with 10^8, 10^6, or 10^4 WHV GEs. Serum WHV DNA became detectable at week 2–6 post-inoculation in the animals receiving 10^8 WHV GEs, while the appearance of serum WHV DNA was delayed to week 10–14 post-inoculation in those receiving 10^6 WHV GEs (Fig. 1A and B). WHV DNA was remained undetectable in Chinese woodchucks receiving 10^4 WHV GEs during the entire experimental period, similar to the mock control animals (Fig. 1C and D). WHcAb was developed in all except one of the animals inoculated with different doses of WHV (Fig. 2). These results indicated that WHV inoculation doses influenced the course of infection in Chinese woodchucks. Thus, the high dose of 10^{8-9} WHV GEs was selected for the inoculation of Chinese woodchucks to test the protective value of NA-based strategies in HBV PEP.

3.2. ETV alone or in combination with a DNA vaccine prevented WHV viremia in Chinese woodchucks

To investigate the protective effect of ETV alone or in combination with a DNA vaccine, the animals were infected with 10^8 WHV GEs and divided into 4 groups: group A was left as infection control receiving no treatment after WHV inoculation; group B received pWHcIm plasmid immunization, group C received ETV treatment, and group D received ETV treatment and plasmid immunization (Fig. 3). WHV DNA and WHV RNA could be detected in the liver as early as 1 h after WHV inoculation (Guy et al., 2008); therefore, ETV treatment was initiated 24 h after WHV inoculation to leave enough time for the virus to infect the hepatocytes.

All of the animals in group A (infection control) and B (pWHcIm immunization) were positive for serum WHV DNA at week 2 after WHV inoculation, 11 of them became negative around week 6–8 and only one animal (1107) remained WHV DNA positive until week 14 after WHV inoculation (Fig. 4A, B, and Table 1). WHV DNA was transiently detected in the untreated animals (group A), except for animal 1107, indicating that 2-year-old Chinese woodchucks regularly developed self-limiting infections after WHV

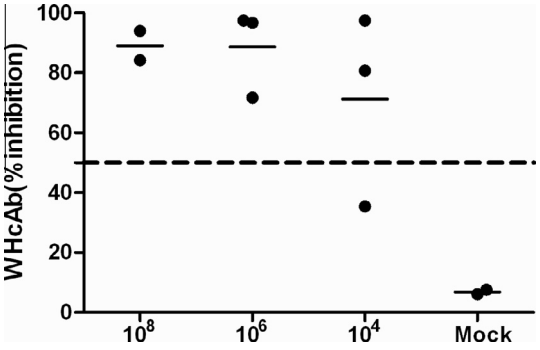

Fig. 2. WHcAb in Chinese woodchucks following inoculation with different WHV GEs. Chinese woodchucks were inoculated with 10^8, 10^6, or 10^4 WHV GEs, respectively. Two animals received saline and served as mock controls. WHcAb was measured by competitive ELISA at week 12 and week 24 post WHV inoculation. The cut-off value is presented by a dotted horizontal line.

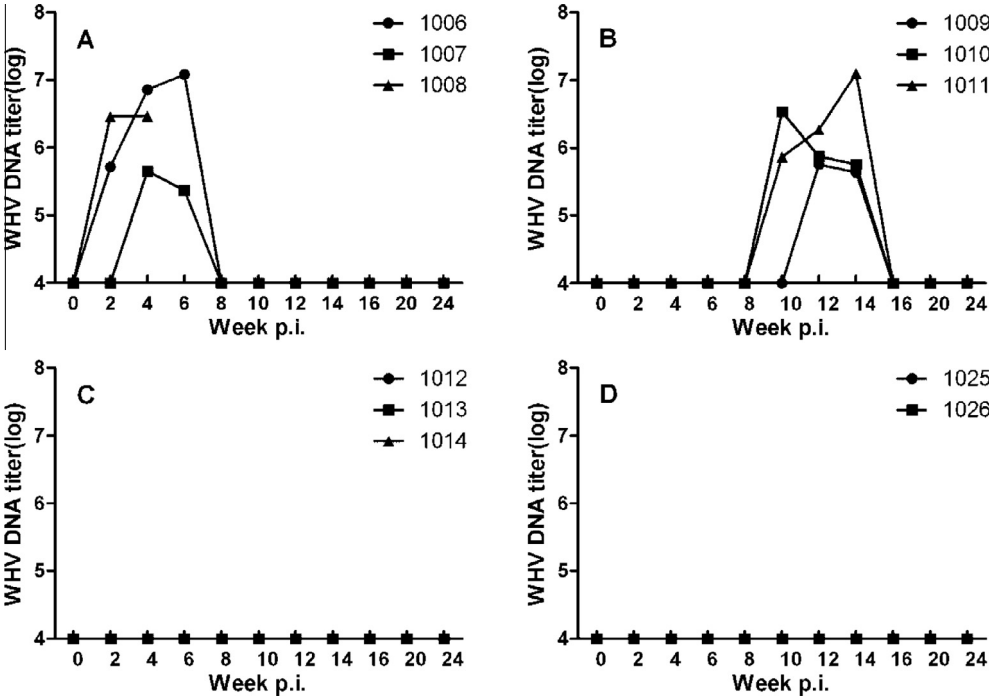

Fig. 1. Viremia in Chinese woodchucks following inoculation with different WHV GEs. Chinese woodchucks were inoculated with 10^8 (A), 10^6 (B), or 10^4 (C) WHV GEs, respectively. Two animals received saline and served as mock controls (D). Blood samples were collected at 2 weeks intervals. WHV DNA was measured by real-time PCR.

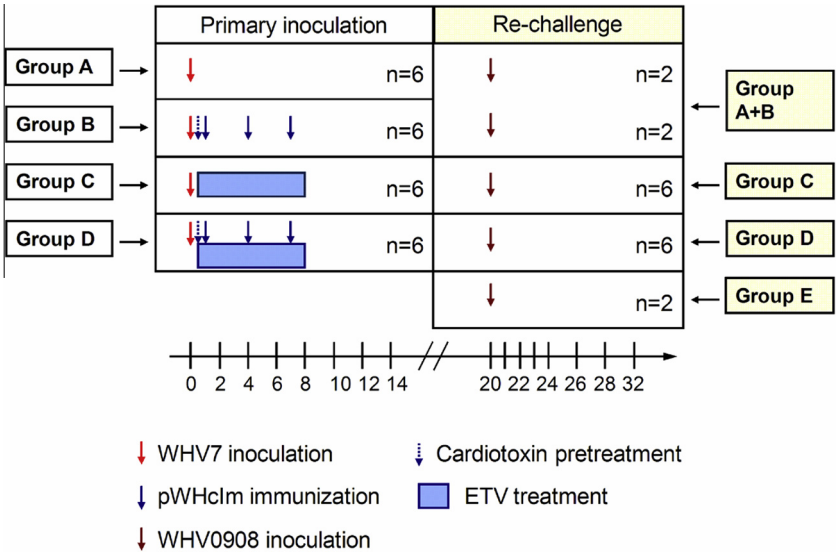

Fig. 3. Experimental design for primary WHV inoculation and secondary re-challenge in Chinese woodchucks. Primary inoculation: Chinese woodchucks were infected (red arrow) with a WHV7 stock with 10^8 GEs and were divided into 4 groups: animals were left untreated and served as infection controls (group A), and were vaccinated with pWHcIm (group B), treated with ETV (group C), or treated with ETV and the pWHcIm vaccine (group D). WHV re-challenge: 20 weeks after primary inoculation, 4 animals of groups A and B (group A + B), and all group C and D animals were re-challenged (brown arrow) with 10^9 WHV GEs. Two naïve Chinese woodchucks were inoculated with WHV and served as re-challenge controls (group E). ETV (blue rectangle) was administered 24 h later and applied continuously for 8 weeks. After the pretreatment with cardiotoxin (dashed blue arrow), animals were immunized pWHcIm (blue arrow) 3 times in 3 week intervals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

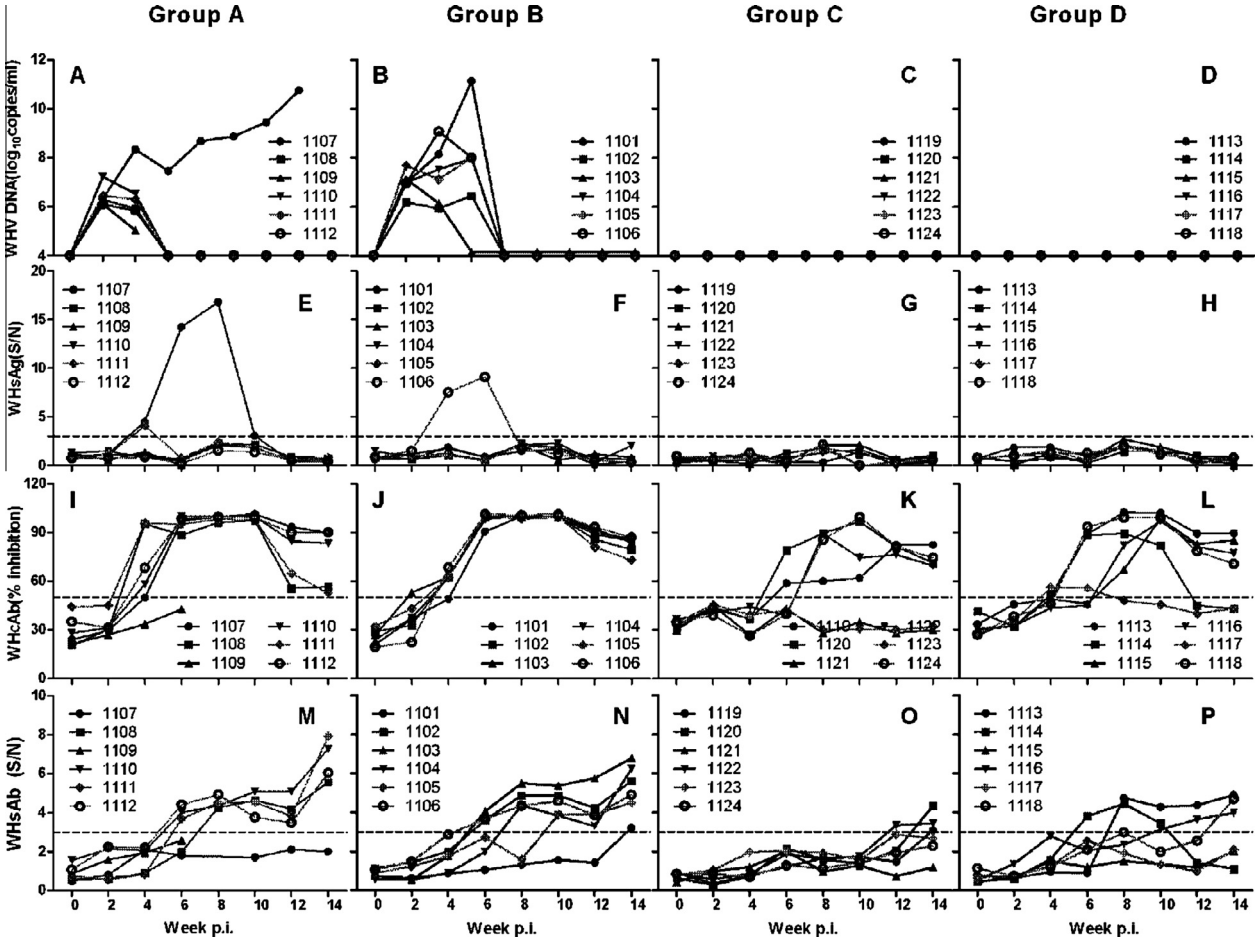

Fig. 4. Kinetics of WHV DNA, WHsAg, WHcAb, and WHsAb following the primary WHV inoculation in Chinese woodchucks. After primary inoculation with 10^8 WHV GEs, group A was left untreated and was used as infection control, other Chinese woodchucks received the pWHcIm vaccine (group B), ETV (group C), or ETV and the pWHcIm vaccine (group D). Serum samples were collected at different time points. WHV DNA (A–D), WHsAg (E–H), WHcAb (I–L), and WHsAb (M–P) were measured by real-time PCR or specific ELISAs. The cut-off value is presented by a dotted horizontal line.

Table 1
Outcome of WHV infection after the primary and the secondary inoculation.

Group	No.	Primary WHV inoculation			Secondary WHV inoculation
		Viremia	WHcAb	WHsAb	Viremia
A	1107	+	+	–	/
	1108	+	+	+	–
	1109	+	/	/	/
	1110	+	+	+	/
	1111	+	+	+	–
	1112	+	+	+	/
B	1101	+	+	+	–
	1102	+	+	+	–
	1103	+	+	+	/
	1104	+	+	+	/
	1105	+	+	+	/
	1106	+	+	+	/
C	1119	–	+	+	–
	1120	–	+	+	–
	1121	–	–	–	+
	1122	–	+	+	–
	1123	–	–	–	+
	1124	–	+	–	–
D	1113	–	+	+	–
	1114	–	+	–	–
	1115	–	+	–	–
	1116	–	+	+	–
	1117	–	+	–	–
	1118	–	+	+	–
E	1146	/	/	/	+
	1147	/	/	/	+

inoculation. These results were similar to previous studies in American woodchucks (Cote et al., 2000). Only 3 (1107, 1111, and 1106) of the 12 animals in group A and B were transiently positive for WHsAg (Fig. 4E, F, and Table 1). All animals except for one (1109) were WHcAb positive at week 6 post-inoculation (animal 1109 died accidentally at week 6), and the level of WHcAb decreased obviously at week 12 post-inoculation in 2 animals of group A, while only a slight reduction was observed in group B (Fig. 4I, J, and Table 1). All animals except for one (1107) were positive for WHsAb at week 14 post-inoculation (Fig. 4M, N, and Table 1). All of the group B animals were not protected from WHV infection, demonstrating that DNA vaccination alone 1 week after WHV inoculation did not induce effective protection from WHV infection in Chinese woodchucks.

In contrast, WHV DNA and WHsAg were not detected following WHV inoculation in animals treated with ETV, whether in the presence (group D) or absence (group C) of pWHcIm immunization (Fig. 4C, D, G, H, and Table 1), indicating that ETV treatment prevented the viremia after the primary WHV infection even with a high dose of 10^8 WHV GEs. This result is consistent with a study of chronically WHV-infected American woodchucks demonstrating that ETV can effectively inhibit WHV replication at doses of 0.5 and 0.1 mg/kg/day (Genovesi et al., 1998). Although WHsAg and WHV DNA were not detected in ETV treated animals during the 8-week treatment period, WHcAb was appeared at 6 weeks post-inoculation, which was 2 weeks later than the infection control group (Fig. 4K and Table 1). Four animals of group C (1119, 1120, 1122, and 1124) became positive for WHcAb (Fig. 4K and Table 1); all of the group D animals were WHcAb positive, though WHcAb positivity in 2 animals (1114 and 1117) was transient and weak (Fig. 4L and Table 1). Only 3 animals of group C (1119, 1120, and 1122) were weakly positive for WHsAb at week 14 post-inoculation, 6 weeks later than those of the infection control group (Fig. 4O and Table 1). Four group D animals (1113, 1114, 1116, and 1118) became positive for WHsAb at week 6–14 post-inoculation (Fig. 4P and Table 1). Of these, animal 1114 became WHsAb negative at week 12 post-inoculation (Fig. 4P and Table 1).

3.3. Chinese woodchucks that received ETV alone or in combination with a DNA vaccine were partially or completely protected from secondary WHV infection

To clarify whether the animals of the group C (ETV alone) and D (ETV in combination with the DNA vaccine) acquired protective immunity after the termination of the treatment, we performed a re-challenge experiment using a new high titer WHV stock containing 10^9 WHV GEs.

Two naïve animals (1147 and 1148) were inoculated with 10^9 GEs of WHV and served as the re-challenge control (group E) (Fig. 3). Serum WHV DNA became detectable in the group E animals at week 2–3 post-inoculation (Fig. 5D and Table 1). Animal 1147 was transiently positive for viral DNA, and WHV DNA and WHsAg remained detectable until the end of the observation period in another animal (1146) (Fig. 5D, H, and Table 1). Both animals were positive for WHcAb at week 28, but negative for WHsAb until the end of the observation period (Fig. 5L and P).

All of the animals of group A (infection control) and B (pWHcIm immunization) except for one (1107) showed self-limiting WHV infection in the primary infection study, therefore only 2 animals from each group (group A + B) were selected to perform the re-challenge experiment (Table 1 and Supplemental Table 2). As expected, neither WHV DNA nor WHsAg were detected in these animals after re-challenge with WHV (Fig. 5A, E, and Table 1). WHcAb remained at the same levels compared to the level prior to the re-challenge in three animals (1101, 1102, and 1111) or was elevated in one animal (1108) (Fig. 5I). However, the level of WHsAb in re-challenged animals from both groups A and B was decreased (Fig. 5M).

WHV DNA was detected in the first week after secondary inoculation in 2 of the 6 group C (ETV alone) animals (1121 and 1123) and remained detectable until the end of the observation period (Fig. 5B and Table 1). WHsAg was detected in these two animals at week 4 post re-challenge (Fig. 5F and Table 1). Thus, these two group C animals did not develop effective protective immunity against the secondary WHV infection. WHcAb was detected at week 4 and 8 post re-challenge, while WHsAb remained negative (Fig. 5J and N). However, WHV DNA and WHsAg were not detectable in the remaining 4 group C animals (1119, 1120, 1122, and 1124) (Fig. 5B, F, and Table 1). WHcAb remained at relatively high levels, and the WHsAb levels fluctuated in 2 animals (1119 and 1120) (Fig. 5J and N).

In group D (ETV in combination with the DNA vaccine), none of the animals were positive for WHV DNA and WHsAg. The WHcAb levels remained unchanged in 4 animals (1113, 1115, 1116, and 1118) or quickly returned to relatively high levels in 2 animals (1114 and 1117), while the WHsAb levels remained unchanged (Fig. 5C, G, K, O, and Table 1).

4. Discussion

In this study, we found that the dose of viral inoculums influenced the course of WHV infection, and the high dose of WHV (10^{8-9} GEs) was selected to inoculate Chinese woodchuck testing the protective value of NA-based strategies in HBV PEP. We also found that both ETV alone and ETV combined with a DNA vaccine could prevent viremia in woodchucks inoculated with high-dose of WHV. However, when ETV treatment was used alone, only 4 of 6 animals were capable of developing protective immune responses against a high dose virus re-challenge, while ETV used in combination with a DNA vaccine effectively protected all of the animals

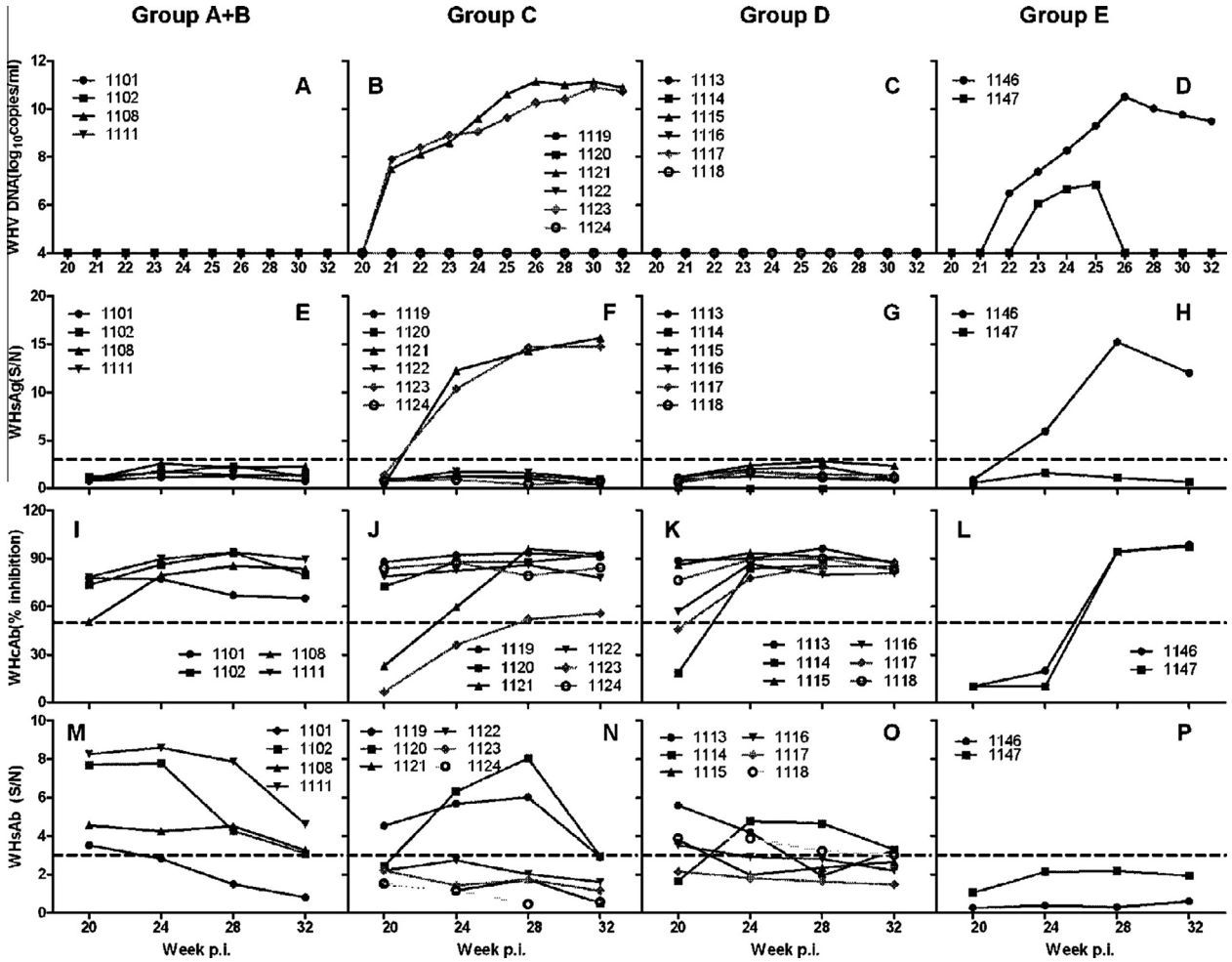

Fig. 5. Kinetics of WHV DNA, WHsAg, WHcAb, and WHsAb following WHV re-challenge in Chinese woodchucks. Twenty weeks after the primary viral inoculation, 4 animals of groups A and B (group A + B), and all group C and group D animals were re-challenged with 10^9 WHV GEs. Two naïve Chinese woodchucks were inoculated with WHV and served as re-challenge controls (group E). Serum samples were collected at different time points. WHV DNA (A–D), WHsAg (E–H), WHcAb (I–L), and WHsAb (M–P) were measured by real-time PCR or specific ELISAs. The cut-off value is presented by a dotted horizontal line.

from the secondary WHV infection even with a high dose inoculation.

In ETV-treated Chinese woodchucks, WHsAg and WHV DNA were not detectable in any of the animals even after the drug treatment was stopped. In addition, low levels of WHsAb were detected in 3 animals (1119, 1120, and 1122) at week 14 post-inoculation. It is well known that ETV, a deoxyguanosine analogue competitive inhibitor of HBV polymerase, can effectively inhibit virus replication, but cannot eliminate the virus (Langley et al., 2007). Therefore, the observed elimination of the virus may be associated with the host's innate or adaptive immune responses. Similarly, none of the animals receiving ETV treatment combined with plasmid pWHcIm immunization after the primary inoculation developed viremia during the complete observation period.

Four of 6 ETV-treated animals remained negative for WHV infection markers within 12 weeks after the re-challenge. Only 3 animals showed low levels of WHsAb following the primary infection, and the WHsAb levels in these animals were not consistently elevated after the re-challenge, indicating that the neutralization effects of WHsAb might not be the determining factor for protection against the secondary challenge. According to the previous studies, the appearance of WHcAb/HBcAb indicates the presence of virus-specific T-cell responses (Lu et al., 1999; Zerbini et al., 2008), and trace amounts of WHV inoculations may also induce WHcAb (Fig. 2) or virus-specific T-cell immune response (Gujar et al., 2013). Taking into account that all 4 animals protected from WHV re-challenge were WHcAb positive following the primary infection, we speculate that a protective role against virus re-challenge might be mediated by virus-specific T-cell responses, while WHcAb positive status suggests the presence of a protective immune response. ETV combined with the plasmid pWHcIm vaccine could effectively protect animals from WHV re-challenge. Previous studies showed that animals vaccinated with the plasmid pWHcIm could develop high titers of WHcAb and effectively protect against WHV infection (Roos et al., 1989; Schodel et al., 1993). The possible mechanism of protection against WHV re-challenge may be associated with pWHcIm-induced immune responses in the animals treated with ETV and the pWHcIm vaccine.

Based on our results, we suggest that NAs might be used for an alternative PEP strategy to prevent HBV infection or at least prevent the viremia. The recommended strategies include (1) NAs combined with vaccine immunization and (2) NAs used alone followed by HBV serological marker screening to determine whether additional vaccination is required or not (Fig. 6). The use of NAs alone could not only replace immunoglobulin injection but also could be advantageous in remote and rural areas, reducing the travel costs of the exposed personal and the transportation and storage costs of HBV vaccine for the government. Furthermore, NAs may also be useful for HBV PEP in the non-responders to the current hepatitis B vaccine. Regarding the cost-effectiveness, the potent NAs with higher genetic

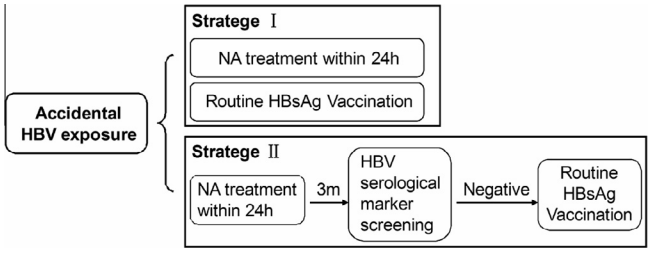

Fig. 6. NA-based strategy for HBV PEP. Two PEP strategies could be applied for high-risk populations following accidental exposure to HBV. Strategy 1: NA treatment is administered within 24 h and routine rHBsAg vaccination is given at 0, 1 and 6 months. Strategy 2: NA treatment is administered within 24 h, and HBV serological marker screening is performed after 3 months. Routine rHBsAg vaccination is applied for those who have a negative antibody response.

barrier, ETV and tenofovir, are preferred in countries where lamivudine (LAM) was used extensively, while LAM could be preferred in countries where LAM was not used extensively. However, the NA-based strategies for HBV PEP need confirmation with further clinical studies and require further optimization, e.g. the dosage and the treatment duration of the selected NAs.

Because of shared transmission routes, co-infection with HIV and HBV is common, an estimated 10% of HIV-infected persons have chronic hepatitis B worldwide (Kourtis et al., 2012; Thio, 2009), and the HCPs may have high risk for suffering HBV and HIV co-infection after the accidental exposure. NAs are currently recommended for HIV PEP, not only for the prophylaxis of occupational exposure in HCPs but also for the prophylaxis of non-occupational exposure such as sexual exposure (Panlilio et al., 2005). Our results indicate that NA based PEP strategy is a promising strategy for HBV PEP. Therefore proper strategy with selected NAs may also be used for PEP of HIV and HBV co-infection, which is supported by two recent retrospective studies. In Japan, among HIV infected men who have sex with men receiving ART, the rate of incident HBV infections was remarkable lower during anti-HBV NAs containing ART than during no ART period and other ART(Gatanaga et al., 2013). In Taiwan, HIV-infected patients receiving lamivudine-containing ART had a lower risk for incident HBV infections (Sheng et al., 2013).

In this study, we tested a NA-based strategy for HBV PEP using the newly established Chinese woodchuck model, and we found that ETV treatment prevented WHV viremia and induced partial protection against a high dose WHV re-challenge. ETV treatment combined with a DNA vaccine prevented viremia after the primary WHV infection and protected the animals from the secondary WHV infection. Based on these results, we suggest that NA-based strategies may be not only a valuable alternative for HBV PEP in those living in the remote and rural areas and the non-responders to the current vaccine but also valuable for PEP of HBV and HIV co-infection, while further clinical studies are warranted to confirm this observation.

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6. Transparency declarations

All authors: none to declare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2014.02.016.

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