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Neutralization of hepatitis B virus (HBV) by human monoclonal antibody against HBV surface antigen (HBsAg) in chimpanzees

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ABSTRACT

The virus neutralizing efficacy of HB-C7A, a human monoclonal antibody raised against the surface antigen of hepatitis B virus (HBsAg), was proved using hepatitis B virus (HBV)-naïve chimpanzees. One control chimpanzee which received 100 CID_{50} of HBV, subtype *adw*, without HB-C7A antibody became infected by HBV as evidenced by the appearance of HBV DNA on week 10 and subsequent appearance of HBsAg, anti-HBc and anti-HBs in the serum. Two experimental chimpanzees were inoculated intravenously with same dose of HBV as the control chimpanzee, which was previously incubated with 0.1 mg and 10 mg of HB-C7A antibody prior to inoculation. HBV infection was not observed in the antibody-treated chimpanzees during 12 months of follow-up, exhibiting neither detectable HBsAg nor anti-HBc antibody. This work demonstrates the neutralization of HBV by HB-C7A monoclonal antibody and shows the possibility of prevention of HBV infection using this antibody in liver transplantation and exposure to HBV.

1. Introduction

Hepatitis B virus (HBV) is one of the principle pathogens responsible for hepatitis and hepatocellular carcinoma, and causes a serious public health problem worldwide (Gitlin, 1997). There are currently 300 million HBV carriers worldwide (Chisari and Ferrari, 1995; Lok, 2000). Chronically infected patients with active liver disease carry a high risk of developing cirrhosis and hepatocellular carcinoma (Chisari and Ferrari, 1995).

Hepatitis B immune globulin (HBIG), prepared from plasma of donors with high anti-HBs antibody titer, is a highly effective prophylactic agent (Heijtink et al., 1999; Keller and Stiehm, 2000). An increasingly important use of HBIG is to prevent hepatitis B recurrence in hepatitis B-seropositive liver transplantation recipients (Keller and Stiehm, 2000). However, the currently available HBIG is not an ideal source of therapeutic antibody due to its limited availability, low specific activity and possible contamination of infectious agents (Ehrlich et al., 1992; Witherell, 2002).

HB-C7A, a fully human monoclonal antibody against a surface antigen of hepatitis B virus (HBsAg), was generated and its immunological and biochemical characteristics were studied (Shin et al., 2007). This HB-C7A antibody exhibits ≥2600 units/mg of antibody which was measured by ELISA based on the WHO (World Health Organization) International Unit of anti-hepatitis B immunoglobulin (International Laboratory for Biological Standards, Amsterdam, The Netherlands) (Shin et al., 2007). In addition the HB-C7A exhibits seven-fold higher affinity than that of Hepabig® (a plasma-derived HBIG from Green Cross Corp., Yongin, Korea) which was estimated by competition ELISA where the HBsAg concentration that gives 50% inhibition of maximum binding (the ELISA reading performed without competitive HBsAg) was determined as affinity; the affinities of HB-C7A and Hepabig® by competition ELISA are $8.0 \times 10^9\ M^{-1}$ and $1.2 \times 10^9\ M^{-1}$, respectively (Shin et al., 2007). Immunohistochemical analysis of HB-C7A antibody to 32 normal human tissues from three unrelated human donors did not demonstrate any tissue cross-reactivity, indicating that this antibody might be safe when it is administered in vivo (Shin et al., 2007). This HB-C7A antibody may have several advantages compared to plasma-derived HBIG such as activity, affinity, safety and availability. The conventional HBIG is required high amount for neutralization of HBV because of its low activity; in contrast this HB-C7A could be applied much less amount considering its high activity.

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Increasing evidence demonstrates that humoral immunity is important for protection from HBV infection. Earlier studies demonstrated that antibodies against the common *a* determinant of the S protein or the pre-S1 peptide neutralized HBV infection in chimpanzees and humans (Hong et al., 2004; Zhang et al., 2006 and references therein; Kim et al., 2008).

Because of the species-specificity of HBV infection, the efficacy of neutralizing antibodies in vivo requires evaluation in chimpanzees, although in vitro models have shown some promise (Zhang et al., 2006 and references therein). In this study neutralization of HBV by this HB-C7A antibody was demonstrated by intravenously inoculating chimpanzees with mixtures of the antibody and HBV for 1 year of follow-up.

2. Materials and methods

2.1. Antibody used in this study

The antibody, HB-C7A, used in this study is a human monoclonal antibody (IgG1, kappa) raised against the surface antigen of hepatitis B virus. This antibody is produced from Chinese hamster ovary (CHO) cells in a bioreactor with serum-free media P1 (Green Cross Corp., Yongin, Korea). Antibody was purified using STREAMLINE rProtein A (GE Healthcare Bio-Sciences, Uppsala, Sweden) from the culture media, then ion-exchange chromatography, and purification of antibody was analyzed by SDS-PAGE. After purification, antibody was analyzed in terms of activity, endotoxin, pyrogen, host cell protein and protein A.

2.2. HBV used for the study

The HBV used for this study was provided by the New York Blood Center, New York (stock no. 78-564). Chimpanzee infectious titer (CID_{50}) of this stock is $1 \times 10^{6.5}$/ml and its subtype is *adw*. The virus was diluted to 100 CID_{50}/ml with 50% normal chimpanzee serum in PBS.

2.3. Animal management

This study was conducted in accordance with the applicable Vilab II standard operating procedures (SOPs) under Good Laboratory Practice (GLP) under the regulatory authority of the US Food and Drug Administration guidelines 21 CFR 200-299. This study received prior review and approval from the Vilab IACUC Committee that adheres to the policies and regulations of the USDA as described in the Animal Welfare Act, 9CFR, Chapter 1. All procedures requiring restraint (injections, obtaining blood samples, weights) were obtained under a combination of ketamine/xylazine.

2.4. Chimpanzee studies

Three tubes, each containing 1 ml of 100 CID_{50} of HBV, were prepared and 0.1 mg of HB-C7A antibody was added to tube no. 1, 10.0 mg of HB-C7A antibody to tube no. 2 and no antibody was added to tube no. 3. All tubes were brought to a total volume of 3.0 ml with antibody and/or PBS. Tubes were inverted to mix and held for 1 h at 37 °C and then overnight at 4 °C.

Each of the HBV-naïve chimpanzees was inoculated intravenously with the contents of one of the three tubes. Three chimpanzees used in this study, chimpanzee 430 (male, 12.2 kg), chimpanzee 435 (male, 11.6 kg) and chimpanzee 436 (female, 10.8 kg), had not been inoculated with any HBV-containing materials and were seronegative for all HBV associated serologic markers. Chimpanzee 430 was infused with 100 CID_{50} of HBV that was preincubated without HB-C7A antibody. Chimpanzees 435 and 436 were infused with 100 CID_{50} of HBV that was preincubated with 0.1 mg and 10.0 mg of HB-C7A antibody respectively.

2.5. Testing of follow-up samples

Blood samples were drawn 1 week prior to administration of test article, weekly from weeks 0 to 8, biweekly from weeks 8 to 24 and monthly from weeks 24 to 51. The long follow-up was necessitated by the long incubation period (30–40 weeks) which can occur following very low dose HBV exposure.

All hematology, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) testings were done in Liberia. Frozen serum and sodium citrate plasma samples were shipped to NY on liquid N_2 and tested for HBV DNA by quantitative PCR. HBV PCR (+) samples were tested for HBsAg. HBsAg (+) animal had samples tested for anti-HBs and anti-HBc until development of antibodies. At the completion of the study, the two experimental animals that received the HB-C7A antibody had a single sample tested for anti-HBs and anti-HBc to verify that they had not been exposed to infectious HBV. All tests for HBV surface markers were performed by EIA.

At 0, 1, 2 and 4 weeks after administration of test article, a serum sample from each animal was submitted to Quest Diagnostics, New York for performance of a Chem-Screen Panel: A/G ratio, albumin, alkaline phosphatase, ALT, AST, direct and total bilirubin, BUN/creatinine ratio, calcium, chloride, cholesterol, CO_2 (bicarbonate), creatinine, gamma glutamyltransferase (GGT), globulin, glucose, iron, lactate dehydrogenase (LD), phosphate, potassium, total protein, sodium, triglycerides, urea nitrogen (BUN) and uric acid.

A catheterized urine specimen was obtained at weeks 0, 1, 2, 3 and 5 and analyzed by microscopic and Chem-Strip 10 urinalysis for examination of sediment and for measurement of specific gravity, leukocytes, nitrate, pH, protein, glucose, ketones, urobilinogen, bilirubin and presence of blood.

All procedures requiring restraint (injections, obtaining blood samples, weights) were obtained under a combination of ketamine/xylazine.

3. Results

3.1. Antibody preparation

HB-C7A monoclonal antibody was produced form Chinese hamster ovary cells as a human IgG1. Protein concentration of the purified antibody was 5 mg/ml. The purified antibody was free of endotoxin and did not contain pyrogen when it was administered to rabbits with 2.5 mg/kg body weight. In addition it did not exhibit abnormal toxicity in guinea pigs when administered with 8.3 mg/kg body weight. The level of host cell protein in purified antibody was below 100 ng/ml and that of protein A was below 10 ng/ml.

3.2. Chimpanzee study

The control animal, chimpanzee 430, that received 100 CID_{50} of HBV without addition of HB-C7A monoclonal antibody developed viremia 10 weeks after inoculation as evidenced by PCR for HBV DNA as could be expected with this dose of HBV (Table 1A). Infection was confirmed by the subsequent appearance of HBsAg, anti-HBc and anti-HBs. HBV DNA remained detectable for 12 weeks (Table 1A). HBsAg was detected at 14 weeks after inoculation and remained positive to week 22. Anti-HBc antibody was detected at 24 weeks after inoculation, remained positive to week 36, and was not measured further (Table 1A). Anti-HBs antibody was detected at 28 weeks after inoculation and remained positive to week 36

Table 1A
Monitoring of HBV markers and transaminases in the chimpanzee 430 which was infused with 100 CID_{50} of HBV that was preincubated without HB-C7A antibody

Study week	ALT (sf units)	AST (sf units)	HBV PCR Log 10 (DNAmol/ml)	HBsAg (EIA/ru's)	Anti-HBs (EIA/ru's)	Anti-HBc (EIA/ru's)
−1	6	11	N[a]			
0	5	10	N			
1	15	13	N			
2	6	16	N			
3	8	22	N			
4	2	6	N			
5	6	11	N			
6	5	12	N			
7	6	20	N			
8	7	18	N			
10	8	18	2.21	.015(−)		
12	10	23	2.43	.023(−)		
14	13	25	3.24	.064(+)		
16	12	18	3.47	.209(+)		
18	8	20	4.10	.600(+)		1.004(−)
20	8	13	4.50	>2.000(+)		1.264(−)
22	10	12	4.82	>2.000(+)	.056(−)	1.038(−)
24	15	18	N	.003(−)	.085(−)	.0129(+)
28	23	22	N	N	>2.000(+)	0.156(+)
32	19	18	N	N	>2.000(+)	0.119(+)
36	21	19	N	N	>2.000(+)	0.061(+)
40	7	23	N	No further testing required		
44	25, 24	19	N			
48	19	16	N			
51	28, 29	23	N			

[a] N denotes negative.

and was not measured further (Table 1A). All of these results were summarized in Fig. 1.

The two experimental animals, chimpanzees 435 and 436, that received mixtures of 100 CID_{50} of HBV and 0.1 mg or 10.0 mg of the HB-C7A monoclonal antibody, showed no sign of HBV infection, remaining PCR negative for HBV DNA during 1 year of follow-up and negative for development of anti-HBc and anti-HBs antibodies (Tables 1B and 1C).

Administration of the monoclonal antibodies at dose up to 10 mg did not result in significant abnormalities in hematology and clinical chemistry, physical examination and urinalysis results, and thus appears to be non-toxic (data not shown).

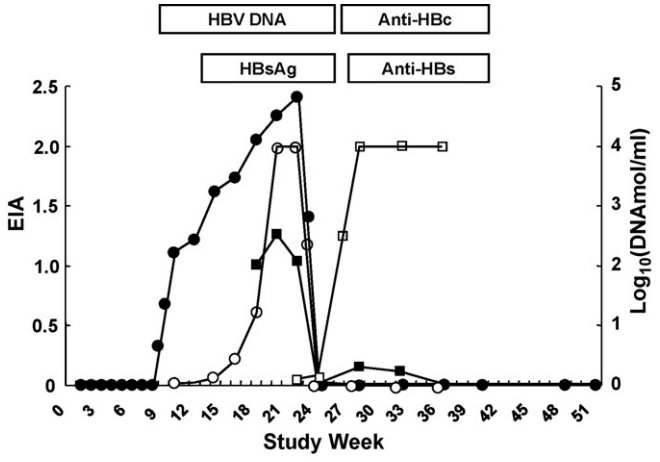

Fig. 1. Course of HBV infection in chimpanzee 430 which received 100 CID_{50} of HBV without addition of HB-C7A monoclonal antibody. HBV DNA (●), which was tested by quantitative PCR, was detected at 10 weeks after inoculation and remained to week 22. HBsAg (○) was detected at 14 weeks after inoculation and remained positive to week 22. Anti-HBc antibody (■) was detected at 24 weeks after inoculation, remained positive to week 36 and was not measured further. Anti-HBs antibody (□) was detected at 28 weeks after inoculation, remained positive to week 36 and was not measured further. All tests for HBV markers were performed by ELISA.

Table 1B
Monitoring of HBV markers and transaminases in the chimpanzee 435 which was infused with 100 CID_{50} of HBV that was preincubated with 0.1 mg of HB-C7A antibody

Study week	ALT (sf units)	AST (sf units)	HBV PCR Log 10 (DNAmol/ml)	Anti-HBs EIA	Anti-HBc EIA
−1	26	22	N[a]		
0	9	26, 25	N		
1	10	23	N		
2	6	24	N		
3	9	25	N		
4	4	18	N		
5	9	37, 37	N		
6	5	25	N		
7	5	9	N		
8	5	13	N		
10	8	17	N		
12	14	21	N		
14	17	23	N		
16	15	19	N		
18	22	16	N		
20	20	16	N		
22	13	19	N		
24	24	22	N		
28	28, 28	25	N		
32	24	26	N		
36	23	25	N		
40	11	20	N		
44	27, 27	17	N		
48	18	13	N	N	
51	30, 29	24	N		N

[a] N denotes negative.

4. Discussion

The HB-C7A antibody exhibits much higher binding activity to HBV compared to the plasma-derived HBIG, recognizes the conformational "a" determinant of HBsAg and binds HBV particles more efficiently than the Hepabig® as proved by immunoprecipitation (Shin et al., 2007). The HB-C7A antibody binds to HBV-infected human liver tissue but does not bind to normal human tissues (Shin et al., 2007). This HB-C7A antibody has several advantages

Table 1C
Monitoring of HBV markers and transaminases in the chimpanzee 436 which was infused with 100 CID_{50} of HBV that was preincubated with 10 mg of HB-C7A antibody

Study week	ALT (sf units)	AST (sf units)	HBV PCR Log10(DNAmol/ml)	HBeAg EIA	Anti-HBe EIA	Anti-HBs EIA	Anti-HBc EIA
−1	5	18	N[a]				
0	11	22	N				
1	7	18	N				
2	5	20	N				
3	13	31, 31	N				
4	9	19	N	(−)	(−)		
5	8	23	N	(−)	(−)		
6	14	26	N	(−)	(−)		
7	7	15	N	(−)	(−)		
8	10	19	N	(−)	(−)		
10	20	16	N				
12	13	19	N				
14	16	21	N				
16	16	24	N				
18	21	24	N				
20	14	22	N				
22	16	19	N				
24	21	17	N				
28	18	21	N				
32	23	16	N				
36	22	17	N				
40	16	23	N				
44	24, 25	15	N				
48	20	19	N			N	
51	28, 31	22	N				N

[a] N denotes negative.

compared to plasma-derived Hepabig® such as activity, safety and availability (Shin et al., 2007).

The goal of this study was to assess the neutralizing activity of HB-C7A human monoclonal antibody against HBV in chimpanzees. Plasma samples were tested for transaminases (ALT and AST), and HBV DNA by PCR to assess infection and viral load. Infection was further confirmed by tests for HBsAg, anti-HBs and anti-HBc. If all of these tests were negative, it was concluded that no HBV infection had occurred. We proved that this HB-C7A human monoclonal antibody was able to neutralize HBV in an in vivo animal model and thus this shows the possibility of applying this antibody for prevention of HBV infection in humans.

ALT and AST levels were not elevated during the HBV infection in the control chimpanzee and exhibited similar levels as the antibody-treated chimpanzees (Tables 1A–1C). Similar results were found in other study (Hong et al., 2004) in which the levels of two enzymes were not elevated meaningfully in an HBV-infected control chimpanzee during a neutralization study (data not shown). In contrast, other studies observed the elevation of ALT and/or AST levels during HBV infection in which 1000 CID_{50} of HBV was inoculated (Iwarson et al., 1985; Zhang et al., 2006).

This study demonstrated only the neutralization of HBV *adw* subtype by HB-C7A antibody. However considering that this antibody recognizes the common "a" epitope (Shin et al., 2007), we predict that this antibody will neutralize the broad spectrum of HBV subtypes. Previous studies demonstrated that antibodies against the common "a" determinant of the S protein neutralized HBV infection in chimpanzee (Ogata et al., 1993) and humans (Eren et al., 2000)

All these results strongly support the desirability of a clinical trial to evaluate the protective efficacy of this monoclonal antibody in preventing infection after liver transplantation and in case of vertical and accidental exposure to HBV.

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