

Hepatic Cirrhosis Occurring in a Young Woodchuck (*Marmota monax*) Due to Vertical Transmission of Woodchuck Hepatitis Virus (WHV)

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Abstract

Histologic and clinicopathologic findings of a woodchuck (*Marmota monax*) vertically infected with woodchuck hepatitis virus (WHV) are presented. The liver exhibits marked cirrhotic changes, which is characteristic of the pre-transformation phase of WHV. At necropsy, the woodchuck exhibited ascites and the liver had a grossly nodular appearance. Microscopically, focal hepatocyte necrosis and inflammatory cells were observed in midzonal and periportal areas in the liver. In Macchiavellos stained sections, cytoplasmic inclusion bodies appeared reddish granular materials. We believe that this may represent a new suitable and cost-effective cirrhotic model for the disease processes associated with hepadnaviruses in a number of other species, most notably Hepatitis B virus infection in man.

Key words: hepatic cirrhosis, woodchuck, woodchuck hepatitis virus

Chronic viral infection has been reported to cause a range of hepatic lesion, including hepatic fibrosis, cirrhosis and hepatocellular carcinoma (HCC) in a wide variety of animal species [14]. In man, Hepatitis B virus (HBV) is a pathogen that causes chronic infection and potentially fatal hepatic disease in millions of individuals, and is associated with a similar spectrum of liver disease [2].

Woodchucks (*Marmota monax*) chronically infected with

woodchuck hepatitis virus (WHV) develop similar progressive hepatic inflammatory and neoplastic lesions that are remarkably similar to those associated with HBV infection in humans [9]. Woodchucks infected with WHV after one year of age may develop hepatic neoplasms in as little of twelve months and the process of malignant transformation is considered to be a model of human hepatic carcinogenesis [6, 12, 13]. Laboratory infections of woodchucks with WHV resemble human and chimpanzee HBV infections both histologically and serologically [6]. Earlier reports focus primarily on the process of carcinogenesis in the woodchuck model, with histologic and serologic comparisons to human and chimpanzee HBV infection.

Transmission of HBV from mothers to babies is the most important route for virus spread within the population. Experimentally, the cases of vertical transmission of WHV were reported in some articles [3, 4]. These reports examined serologically and/or detected WHV DNA in the liver and lymphatic tissue [3, 4]. There was investigated correlation with HCC and WHV infection [7, 12].

In this report, we encountered a woodchuck case of naturally vertical infection with WHV. Thus we concentrate on the cirrhotic model of WHV infection with specific regard to biochemistry and histopathology.

One two-month-old offspring was obtained from twenty female woodchucks (*Marmota monax*) experimentally infected with WHV purchased at Northeastern Wildlife Inc. (South Plymouth, NY, USA). These mother woodchucks were housed individually or in pairs in wooden nest boxes with woodshavings and wheat straws provided as nesting materials. The animals were maintained on twelve-hour light and dark cycles and fed commercially available laboratory rabbit chow (Purina Mills LLC., St. Louis, MO, USA) and water ad libitum. One offspring of woodchuck was autopsied and blood, serum and a range of tissues submitted for clinico-

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pathologic and histopathologic analysis.

Anticoagulated blood and serum were collected for analysis of the following components - aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl transferase (γ -GT), alkaline phosphatase (ALP), creatinine, cholesterol, triglyceride (TG), blood urea nitrogen (BUN), uric acid, total protein (TP), and albumin.

Liver tissue of woodchuck was removed for histologic examination. After fixation in 10% neutral buffered formalin, liver tissue was embedded in paraffin and cut into 2 ~ 3 m section. Liver sections were stained with hematoxylin-eosin (H&E) and Azan. Additionally, serial liver sections were examined with orcein reaction [1] and Macchiavellos stain methods [8] to detect intracytoplasmic inclusion body of WHV. This orcein reaction of Shikatas technique was used routinely to inclusion body of hepatitis virus and modified by others [7]. For immunohistochemical staining, liver sections were stained using α -smooth muscle actin (α -SMA) antibody (Sigma-Aldrich, St. Louis, MO, USA).

In the infected woodchuck, levels of AST, γ -GT, creatinine, cholesterol, TG, and BUN were significantly increased compared to those of normal woodchucks (Table 1), suggesting hepatic damage in this vertically infected individual.

At necropsy, the woodchuck exhibited ascites and the liver had a grossly nodular appearance (Fig. 1-1). Microscopically, focal hepatocyte necrosis and inflammatory cells were observed in midzonal and periportal areas in the liver.

Orcein-positive inclusions in hepatocytes were detected as markers of HBsAg (Hepatitis B virus surface antigen). In Macchiavellos stained sections, cytoplasmic inclusion bodies appeared reddish granular materials (Fig. 1-2). In this study, we performed specially Macchiavellos stain, which used for detection of rickettsia or chlamydia, because of more simple and evident staining results. Hence, the stain may be a useful and simple screening technique to HBsAg in wood

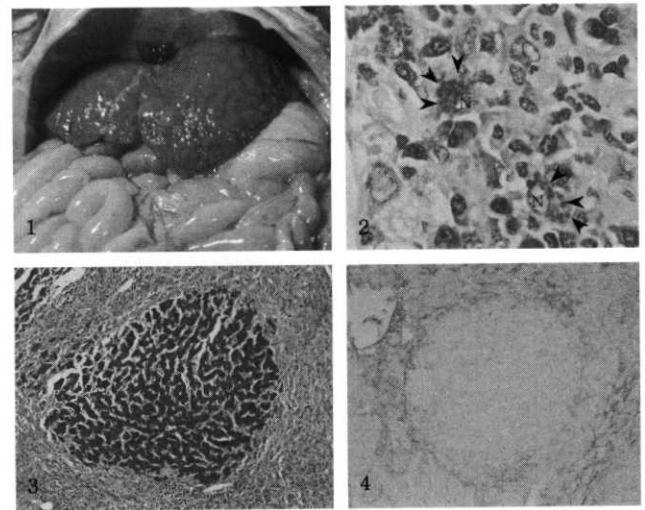


Fig. 1. Irregular nodules were observed grossly in the liver of woodchuck (1). Distinct intracytoplasmic inclusion bodies (arrowhead) were observed in the hepatocytes (2). Macchiavellos stain, $\times 330$. Collagen fibers were deposited in the fibrotic tissue of pseudolobule. Azan stain, $\times 132$. Immunohistochemically positive cells of α -smooth muscle actin (α -SMA) were detected in fibrotic tissue of pseudolobule. Immunohistochemical stain, $\times 132$.

chuck infected with WHV.

Bridging portal fibrosis produced pseudolobulation due to entrapment of hyperplastic hepatocytes (Fig. 1-3). Biliary hyperplasia, ductular cell proliferation, and increased amounts of fibrous connective tissue expanded toward portal areas and extended into other periportal areas. Myofibroblasts stained positive for α -SMA were detected in proliferating fibrotic tissue and sinusoids (Fig. 1-4). These hepatic lesions in this vertically infected animal were grossly and micros-

Table 1. Biochemical values of normal and woodchuck hepatitis virus-infected woodchuck

Parameter ^{a)}	Unit	Normal woodchuck	Infection woodchuck	Parameter ^{a)}	Unit	Normal woodchuck	Infection woodchuck
AST (0~40) ^{b)}	IU/l	18	223	BUN (10~26)	mg/dl	20	135
ALT (0~40)	IU/l	1	18	Creatinine (0.7~1.4)	mg/dl	0.7	2.1
γ -GT (11~63)	IU/l	1	85	Uric acid (3~7)	mg/dl	0	0.1
ALP (60~300)	IU/l	31	29	TP (6~8)	g/dl	5	7.5
Chol (0~240)	mg/dl	167	568	Albumin (3.3~5.2)	g/dl	2	1.3
TG (0~200)	mg/dl	168	384		g/dl		

a) AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ -GT, γ -glutamyl transferase, ALP, alkaline phosphatase; Chol, cholesterol; TG, triglyceride; BUN, blood urea nitrogen, TP, total protein.

b) Normal reference range.

copically consistent with hepatic cirrhosis, and included irregular macro- and micro-nodular hyperplasia, biliary hyperplasia, and cholangiofibrosis. Positive immunostaining for α -SMA suggested the presence of myofibroblasts or their precursors, and hepatic stellate cells [5]. Earlier studies report that naturally infected *Marmota monax* has been validated as a model to study the pathogenesis and clinical course of chronic hepatitis B virus infections in humans [7]. In early stages, WHV is associated with chronic hepatitis and steatosis, progressing to hepatic fibrosis and cirrhosis, and ultimately primary hepatocellular carcinoma [10, 11].

Based on these preliminary findings, we believe that vertically infected woodchucks may provide a cost-effective animal model for the study of early inflammatory and cirrhotic changes associated with HBV in man, and other pathogenic hepadnaviruses. Further investigation of this promising model might be warranted.

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