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HBV and HDV replication in experimental models: effect of interferon

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Abstract

The use of HBV and HDV experimental models has significantly contributed to understand the viral life cycle and to systematically test antiviral effects of various drugs on a pre-clinical level. Similar replication strategies of related hepadna viruses permit the use of chimpanzees (*Pan troglodytes*), woodchucks (*Marmota monax*), ground and tree squirrels (*Spermophilus beecheyi*) or Pekin ducks (*Anas domesticus*) as appropriate animal models. Cell culture systems for in vitro infection or transfection using both primary cultures of human and non-human hepatocytes and non-hepatocytes and cell lines have recently been identified. The advantages and restrictions of these experimental models with respect to evaluation of interferon effects on viral and hepatocellular gene expression are discussed.

Key words: HBV; HDV; Experimental model; Cell culture; Interferon

1. Introduction

The past decade has considerably increased the understanding of Hepatitis B (HBV) and Hepatitis Delta Virus (HDV) infection, replication and pathogenesis. Various antivirals have been used to treat chronic viral infection and interferons (IFN) represent to date the most promising therapeutic approach. In this review, we summarize relevant HBV and HDV experimental models that were used to investigate the viral life cycle and to study interferon effects on virus replication and gene expression.

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2. The hepatitis B virus

Together with the woodchuck hepatitis virus (WHV), the ground (GSHV) and tree squirrel hepatitis virus (TSHV), the duck hepatitis B virus (DHBV) and the heron hepatitis virus (HeHV) HBV makes up a group of small, enveloped hepatotropic and host-specific hepadna viruses, which contain circular, partially double-stranded DNA (for a review see Ganem and Varmus, 1987). The 42 nm infectious virion called Dane particle consists of a nucleocapsid and a lipoprotein envelope. The latter is built of three different types of HBV surface proteins, LHBS, MHBS and SHBS, which are inserted in a lipoprotein bilayer of cellular origin. The nucleocapsid contains the viral DNA, a polymerase with reverse transcriptase and RNase H activity and a genome-bound protein (Lau and Wright, 1993).

The complete genetic information is encoded in 3.2 kB of viral DNA. The L(-) strand of the genome carries five overlapping open reading frames, preS1/S2/S, preC/C, polymerase, X and ORF5 which encode at least seven proteins. On the S(+) strand there are several short open reading frames for which neither a gene product nor a function has been shown so far (Kaneko et al., 1988; Miller et al., 1989). In addition to four promoters (S1-promoter, S2-promoter, C-promoter, X-promoter) and a polyadenylation site the HBV genome carries two enhancers (ENH I and II), a glucocorticoid-responsive element as well as a binding site for nuclear factor-1 (Ganem and Varmus, 1987; Lau and Wright, 1993).

The study of related hepadna viruses that infect non-primates has elucidated the mode of hepatitis virus replication. Similarly to DHBV (Mason et al., 1982) the HBV DNA genome replicates asymmetrically in the infected hepatocyte (Seeger et al., 1986; Will et al., 1987). After the protein bound to the 5'-end of the L(-) strand is removed, both DNA ends are ligated. The S(+) strand is completed by an endogeneous DNA polymerase in the nucleus giving rise to a covalently closed circular HBV DNA. A cellular DNA-dependent RNA polymerase II synthesizes the 3.5 kB pre-genome of (+) strand polarity starting from the HBC-promoter. The pre-genome is packaged in nucleocapsids which are translocated to the cytoplasm. In these nucleocapsids the synthesis of the L(-) strand takes place by an HBV polymerase using a protein primer at the Direct Repeat 1 region (Bartenschlager et al., 1988). Simultaneously, the pre-genome is degraded by an RNase H activity of the HBV polymerase leaving only a short oligoribonucleotide at the 5'-end. This will hybridize with the Direct Repeat 2 region and initiates the S(+) strand synthesis. Due to a single strand nick the synthesis terminates at the 5'-end of the L(-) strand and is only continued after an intramolecular template switch to the 3'-end of the L(-) strand. During this procedure the S(+) strand is not completely synthesized giving rise to the partially double-stranded HBV genome with the characteristic single-stranded gap region. The nucleocapsids are coated with HBV surface proteins and cell membrane lipids in the endoplasmic reticulum and are secreted as complete Dane particles into the blood.

3. The hepatitis delta virus

HDV is a defective virus of approximately 38 nm size that requires HBV or WHV surface proteins as an envelope for the completion of infectious particles (Rizetto et al., 1983). Molecular studies of the 1.7 kB RNA genome have shown a circular conformation and the ability to fold by intramolecular basepairing to form an unbranched rod structure (Wang et al., 1987; Kos et al., 1986). The HDV genome codes for at least one polypeptide that bears the Hepatitis Delta antigen (Weiner et al., 1988) which interacts with HDV-RNA and the HBV envelope proteins in the assembly of viral particles. It plays a crucial role in the replication of HDV RNA. Coinfecting HBV is dispensible for the replication of HDV RNA within an infected cell (Sureau et al., 1991). HDV replication involves the copying of the genomic RNA into a complementary antigenomic RNA which in turn acts as template for the synthesis of more genomic RNA. The circular conformation of intracellular RNAs suggest replication by a "rolling circle model" (Hutchings et al., 1986). Some features of HDV RNA like intramolecular basepairing and the ability to autocleave itself (Kos et al., 1986; Sharmeen et al., 1988; Wu and Lai, 1989; Taylor et al., 1987) have been found to resemble viroids.

4. HBV and HDV experimental models

A large variety of cell culture systems and animal models are today available to study HBV and/or HDV replication, virus-host interactions and the effects of different antivirals. Similar biochemical properties of hepadna virus DNA polymerases permit to use ducks, woodchucks and ground squirrels as experimental models to study the effect of antivirals on hepatitis virus infection (Hantz et al., 1984). Most conventional inhibitors of DNA synthesis interfere with cytoplasmic viral DNA synthesis, but do not eliminate nuclear DNA. Therefore, virus production often reaches pre-treatment levels, when the drug administration is stopped. In addition, HBV DNA can integrate into the hepatocyte genome during almost every stage of infection (reviewed by Rogler et al., 1987) and viral protein synthesis can be mantained from integrated HBV DNA. Interferons are considered useful in HBV or HDV infection, since they are capable to inhibit viral protein synthesis, to enhance the turnover of viral RNA and to exert immunomodulatory effects, e.g., by stimulating cytotoxic T-lymphocytes to clear HBV infected hepatocytes. Interferons and other antiviral drugs have been successfully tested in the WHV, DHBV and GSHV model. These systems are neither easy to handle nor do they substitute completely for the infection of humans. Ducks appear to be less sensitive to toxic drug effects then woodchucks, which is explainable with the different drug metabolism in these two species (Cova et al., 1993). Pre-testing of FIAU, a fluorinated pyrimidine with potent antiviral activity, in the animal model did not help to recognize severe side effects like mitochondrial injury, lactic acidosis and subsequent hepatic failure that became only apparent, when clinical pilot trials were performed (J.H. Hoofnagle and C. Lopez, 1993; unpublished data). A nude mouse model for the in vivo produc-

tion of HBV (Zhai et al., 1990) or transgenic mice models (Yamamura et al., 1990; Chisari et al., 1986 and 1989) have been described, but are in general more useful to study tumorigenesis or immunopathogenesis. The chimpanzee model, however, has played an important role for the understanding of HBV and HDV replication, for the development of serologic tests, the characterization of viral constituents and for molecular cloning (Purcell et al., 1987). Coinfection of the suceptible chimpanzee with HBV and HDV results in clinical manifestation of hepatitis with HBcAg (hepatitis B core antigen) and HDAg (hepatitis delta antigen) expression in hepatocytes. A sequential expression of these antigens is also possible. Superinfection of HDV in chronic HBV carriers tends to result in severe hepatitis. HDV superinfection suppresses HBV replication and antigen expression and can result in the development of chronic HDV infection. These clinical similarities and the pronounced cross reactivitiy between human and chimpanzee immunoglobulins permit to use the chimpanzee model for the development of serologic assays and the testing of antiviral drugs. However, the limited availability and great expense to keep these animals make the chimpanzee a useful experimental model only for a restricted number of research laboratories.

Recently cell culture systems for in vitro infection or transfection and replication of hepadna viruses have been reported, which can be used to investigate the efficacy of antiviral therapies. Both primary cultures of human (Rijntjes et al., 1989; Gripon et al., 1988; Ochiya et al., 1989) and non-human (Jacob et al., 1985; Shih et al., 1990) hepatocytes and non-hepatocytes (Galun et al., 1992; Colucci et al., 1988; Milovanovic et al., 1987) and cell lines (Ueda et al., 1989; Tsurimoto et al., 1987; Hayashi et al., 1989; Tong et al., 1991; Sells et al., 1988; Gerber et al., 1988; Chang et al., 1987; Sureau et al., 1986; Yaginuma et al., 1987) are available (Fig. 1). They have all been shown to support viral replication and gene expression. For HDV, primary chim-

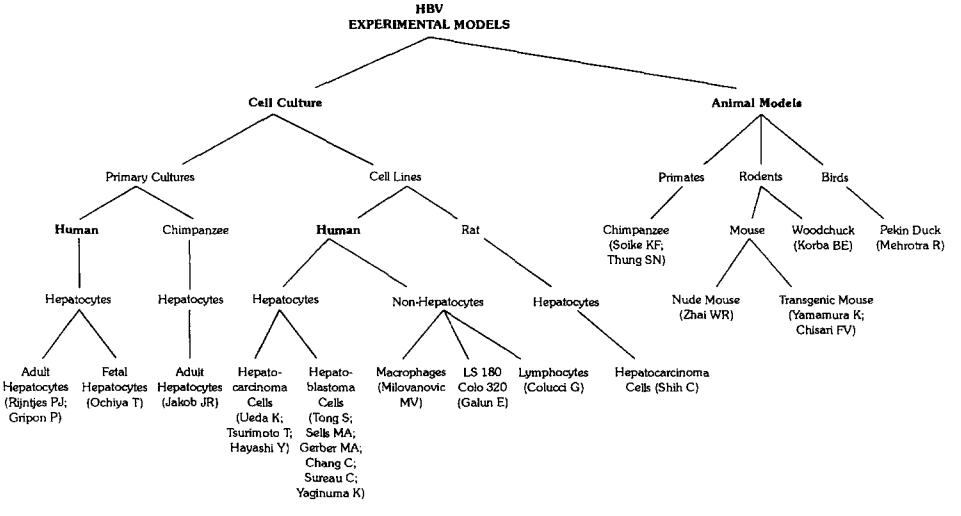

Fig. 1. Overview on HBV experimental models classified in cell culture systems and animal models. Human cells in bold printing.

panzee (Sureau et al., 1991) or woodchuck hepatocyte cultures (Rasshofer et al., 1990; Taylor et al., 1987; Choi et al., 1989) as well as the human cell lines HuH7 (Wu et al., 1991), H1δ9 and HepG2 (Gowans et al., 1991) have been used. While the nucleoside analogon ribavirin was shown to inhibit HDV replication at a concentration of 10 μg/ml, if added by 3 days after infection, suramin was only able to inhibit HDV replication, if added simultaneously with the virus at a concentration of 200 μg/ml, which was not toxic to primary woodchuck hepatocytes (Choi et al., 1989). When HepG2 cells were transfected with a plasmid containing a trimer of HDV and subsequently treated with 20 or 100 units/ml of IFN-α 1 to 7 days after transfection, no effect of IFN-α on HDV genome replication was seen. The authors conclude that HDV may be resistent to interferon treatment in the absence of HBV (Ilan et al., 1992).

For the investigation of the effect of interferon in cell culture on HBV gene expression and replication, the most widely used model systems are hepatocarcinoma (Ueda et al., 1987; Tsurimoto et al., 1987; Hayashi et al., 1989) and hepatoblastoma cell lines (Tong et al., 1991; Sells et al., 1988; Gerber et al., 1988; Chang et al., 1987; Sureau et al., 1986; Yaginuma et al., 1987). In our own experiments a hepatoblastoma cell line transfected with HBV DNA (HepG2.2.15; Sells et al., 1987) was used to study the effects of various interferons on HBV replication and hepatocellular gene expression (Caselmann et al., 1992). IFN-α2B or IFN-β inhibited HBV replication transiently as shown by a significant reduction of HBV replicative DNA intermediates <3.2 kB (Fig. 2). Three days after exposure to IFN the amount of free

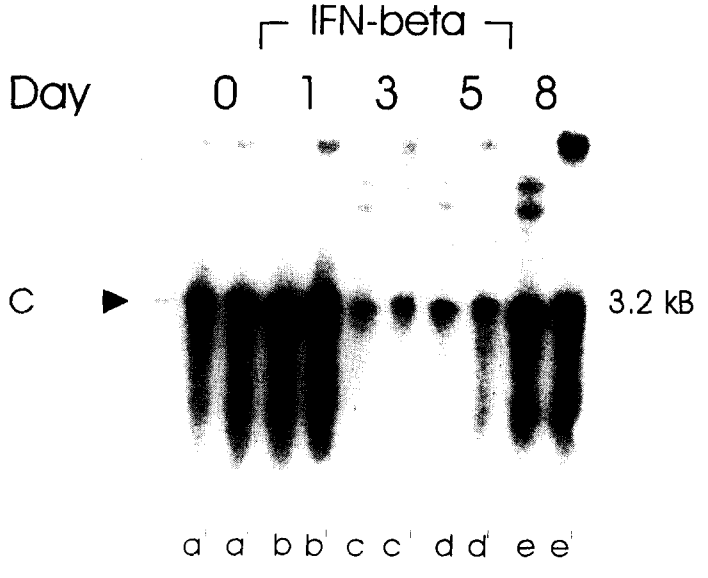

Fig. 2. Southern blot analysis of uncut (a',b',c',d',e') and Hin*d*III-digested total HepG2.2.15 DNA (b,c,d,e) using ^{32}P-dCTP-labeled full-length HBV DNA as a probe. Numbers reflect days. The treatment period was 5 days (1–5) with 1×10^4 U/ml IFN-β. C: 10 pg of linearized cloned HBV DNA as a control. Size marker in kB at the right.

HBV DNA decreased and remained suppressed during IFN application. However, 1 day after the removal of IFN from the medium HBV replication re-occured. In parallel, there was a decrease in the amount of HBV mRNA. The hepatitis B surface antigen and early antigen secretion were not influenced, however their intracellular levels diminished during treatment. The cellular 2'-,5'-oligoadenylate synthetase activity was increased 9- to 18-fold during treatment of cells with IFN-γ, IFN-α or IFN-β. The number of IFN-α and IFN-β receptors was down-regulated, while the number of IFN-γ receptors remained constant during treatment. The expression of major histocompatibility complex class I (MHC class I) antigens was stimulated by addition of IFN-α or IFN-β. The data obtained with IFN-α or IFN-β is consistent with that from other groups who observed a transient dose-dependent IFN effect on HBV DNA in HB611-cells (Ueda et al., 1989) or in core particles derived from Hep-HB107 cells (Hayashi et al., 1989). In contrast to our findings no decrease in the amount of the 3.5 kB transcript was seen after 3 day IFN incubation in the latter study. This may have been due to the short period of IFN exposure in those experiments or could reflect cell-specific differences between the cell lines used. However, preliminary data from the same group obtained in a transient expression system indicated that concentrations of 10^4U/ml IFN-α reduced the 3.5 kB HBV mRNA transcript by 88%. The only other study using HepG2.2.15 cells focused on the analysis of intracellular secreted HBV DNA after exposure to various antivirals (Lampertico et al., 1991). Incubation of the cells with 10^4 U/ml IFN-α resulted in a 45% reduction of the extracellular HBV concentration and a 20% cytotoxicicy rate. In our experiments IFN-γ did not exert a measurable effect on HBV replication and viral protein expression in concentrations of 1×10^1-10^5 U/ml which may be explained by the known lower antiviral potential of IFN-γ, but could also be due to the use of different cell lines. The enhanced MHC class I antigen A2, A9, B5 and B17 expression observed after cells were exposed to IFN type I indicates that immunomodulatory effects may play an important role in mediating the antiviral activities of IFNs. The main shortcoming of the cell culture system is that extrahepatocyte effectors (e.g., cytotoxic T-lymphocytes) and HBV-specific antibodies that may be crucial for proper immune response in vivo are missing. Nevertheless, the cell culture experimental model is a useful tool for assessing antiviral effects on HBV replication and hepatocellular or viral gene expression. It provides insights into the function of the IFN system and may help to define future therapy strategies on a pre-clinical level.

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