MINIREVIEW

Possible Role of Retinoids in Hepatitis B Virus-Associated Liver Damage

ANTHONY R. MAWSON*,[1] AND TIMOTHY A. STEELE†

*Public Health Program and †Department of Microbiology, Des Moines University Osteopathic Medical Center, 3200 Grand Avenue, Des Moines, Iowa 50312

Liver damage following hepatitis B virus (HBV) infection may be due to the action of retinoids as modulators of viral replication. The reduced rate of survival of liver grafts in patients with HBV infection could also be due to the continued presence of the virus, stimulated by retinoids in the graft tissue. Subject to obtaining empirical support for this hypothesis, the use of retinoid-blocking agents could be explored to reduce the risk of liver damage in HBV infection and to enhance the survival of liver grafts. Continued use of such agents may need to be used in conjunction with anti-viral modalities such as HBV hyperimmune globulin and lamivudine to prevent recurrent liver damage and to increase the long-term viability of the graft.

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Hepatitis B virus (HBV) is a nonenveloped, double-stranded DNA virus that has worldwide distribution. With over 300 million chronic hepatitis B carriers globally, HBV infections are an important contributor to morbidity and mortality in humans (1). Infection with HBV generally takes place through a parenteral or maternal-to-infant mode of transmission. Those considered to be at risk for infection include infants born to HBV-infected mothers, dialysis patients, male homosexuals, drug addicts, health care workers, and individuals receiving blood or blood products (2, 3). Approximately one-half of all infections result in subclinical manifestations or mild, nonspecific symptoms, whereas 1% of all infections result in fatal fulminant hepatitis. A unique clinical and histological syndrome seen in about 20% of cases of recurrent HBV infection is fibrosing cholestatic hepatitis (FCH). Clinical features include severe cholestasis and hypoprothrombinemia, progressive hepatic failure, and death in most patients (4). Nearly 10% of infected adult patients go on to develop chronic HBV infection, defined as infection of longer than 6 months, and one-half of all neonates infected at birth become chronic carriers. In the chronic-carrier state, progressive liver destruction may or may not occur (5). Some HBV patients, after incubation periods of up to 40 years, develop hepatocellular carcinoma infection (6). HBV is not cytopathic, and destruction of hepatocytes is thought to occur via an immunologic response to viral antigens. Indeed, cytotoxic T lymphocytes have been isolated that may be involved in damaging hepatocytes (7, 8). Since acute hepatitis B generally resolves spontaneously, only severe cases warrant supportive care. Chronic hepatitis may be treated with interferon-alpha, which induces remission in some patients by upregulating the expression of class I major histocompatibility (MHC) proteins to promote recognition of viral proteins by cytotoxic T lymphocytes (9–11). In severe cases, liver transplantation may extend the lifespan of patients with progressive liver failure (Fig. 1).

Over 4,000 liver transplants are performed annually in the United States. Currently, hepatitis caused by non-A, non-B hepatitis viruses is the most common indication for liver transplantation in adults. However, liver transplantation has been performed for other clinical conditions, including hepatitis B-induced cirrhosis. While transplantation is standard therapy for end-stage chronic liver disease or fulminant liver failure, HBV infection can be especially devastating to liver transplants. The 1-year survival of patients with cholestatic or alcoholic liver disease in most transplant centers is greater than 90%. In contrast, prior to current anti-viral therapies, survival in patients transplanted

[1] To whom requests for reprints should be addressed at Des Moines University Osteopathic Medical Center, 3200 Grand Avenue, Des Moines, IA 50312. E-mail: Anthony.Mawson@dmu.edu

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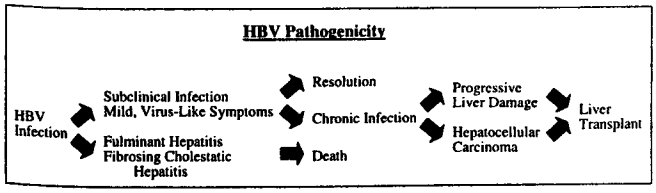

Figure 1. HBV infection leads to variable disease outcomes, and not all patients experience all of the above manifestations. However, for more severely affected patients, liver transplantation may offer the only life-saving treatment option.

for HBV infection was only 50% (12). To reduce the recurrence of hepatitis and cirrhosis, hepatitis B hyperimmune globulin and antiviral agents such as lamivudine can be administered to the liver transplant recipient. These modalities significantly improve the outcome of liver transplantation for hepatitis B (13).

Why is HBV infection so devastating in liver transplant recipients? As noted above, it is felt that liver damage in immunocompetent patients is largely immune mediated, and that MHC-restricted cytotoxic T cells recognize viral peptides in association with class I MHC molecules on the target hepatocyte. Intrahepatic expression of viral proteins is higher in patients with FCH than in transplant recipients without FCH, as well as in nontransplanted patients with chronic HBV infection (14). Since HBV is not cytopathic, the accumulation of viral proteins within the endoplasmic reticulum could be directly cytotoxic, as suggested by experiments with transgenic mice genetically manipulated to overproduce HBV core antigen; hepatocyte necrosis and even hepatocellular carcinoma are produced in the absence of a T-cell response (15). High level accumulation of HBV core antigen has also been shown to be cytopathic in human hepatoma cell lines (16).

Retinoids and HBV Infection

The model that we are proposing states that liver damage following HBV infection—and the reduced rate of survival of liver grafts in patients with HBV infection—is due in part to the action of retinoids as cellular toxins. Since retinoids can accumulate in the cholestatic liver, it is conceivable that a direct toxic effect of retinoids on hepatocytes participates in the liver destruction seen during HBV infection. In addition, as will be seen below, retinoic acid receptors may play an important role in HBV replication, providing a potential anti-viral target for a therapeutic approach to HBV infection.

Retinoids are dietary-derived, fat-soluble signaling molecules that are stored in the liver and are present mainly in the form of retinol (vitamin A alcohol), retinyl palmitate and stearate (esters), retinal (aldehyde), and retinoic acid (the major metabolite). Retinoic acid (RA) is much more biologically active and hence potentially toxic than retinol. Retinoids play a double-edged role in biological systems. At low concentrations, they are essential in numerous biological functions, including normal cell homeostasis, differentiation and growth, embryonic development, vision, and mucus secretion (17, 18), whereas at higher concentrations they inhibit cell growth, act as cellular toxins, and can be extremely mutagenic (19, 20). The nonparenchymal Ito (or stellate) cells in the liver contain about 90% of the total vitamin A reserves of the body, the predominant storage form being retinyl ester (palmitate); smaller amounts are found in hepatocytes within lipid droplets and in the kidney, lungs, and adrenal glands.

RA exerts its effects through binding to nuclear receptors, of which there are two types: RA receptors (RAR) and retinoid X receptors (RXR). RARs and RXRs exist as three distinct gene products: α, β, and γ. RARs bind all-*trans*-RA (tretinoin) and 9-cis-RA, whereas RXRs only bind 9-cis-RA. These transcription factors regulate their expression by forming homodimers (RXR/RXR) or heterodimers (RXR/RAR), and subsequent gene expression occurs as a result of RXR/RAR binding to two directly repeated consensus motifs (AGGTCA) separated by 1, 2, or 5 nonspecific nucleotides (21).

Retinoid toxicity is associated with high-to-normal or even low circulating retinol levels, together with increased fractions of retinyl esters circulating with plasma lipoproteins and not bound to retinol-binding protein (RBP) (22). Retinyl esters react more randomly with the membranes of cells than the physiologically sequestered retinol bound in holo-RBP and hence are a major form of vitamin A toxicity (23) (Table I). Hypervitaminosis A occurs most commonly due to self-medication. Following excessive intakes, the liver becomes saturated with vitamin A and considerable amounts of retinyl esters spill over into the circulating blood

Table I. Symptoms and Signs in Hypervitaminosis A[a]

Symptoms/signs
Increased cerebrospinal fluid pressure and associated neurologic changes, including headache, confusion, irritability, etc.
Fatigue
Hepatomegaly
Hepatic fibrosis and cirrhosis
Portal hypertension
Palmar erythema
Spider angiomas
Ascites
Splenomegaly
Gastrointestinal symptoms
Joint pains
Bone Pain
Osteoporosis/osteopenia
Calcification of soft tissue
Muscle pain
Keratoconjunctivitis (dry eyes)
Xerostomia (dry mouth)
Pruritus
Xanthomas
Excessive dryness of skin
Alopecia (loss of hair)

[a] References: 25, 30, 33, 54, 55, 56.

(Table II). In hypervitaminotic humans, retinyl ester values can be as high as 67% of the total plasma vitamin A (24). Endogenous and/or administered RA (and other acidic retinoids), although not forming similar esters, is considerably more biologically active and more toxic than retinol itself (25). Serum retinol levels may be normal or low because administered RA reduces plasma retinol levels via feedback inhibition (26). A single oral dose (0.167 mmol) of all-*trans*-RA results in an immediate 20% drop in serum retinol that continues for 24 hr (27). Demonstration of increased hepatic stores, along with increased serum retinyl ester and/or RA, is, therefore, essential in determining retinoid toxicity.

Retinoid toxicity can also occur endogenously in association with cholestasis. As noted, HBV infection is associated with cholestasis and occasional liver failure (12). It has been known for many years that in HBV infection, transthyretin and plasma vitamin A are decreased (28) in conjunction with high plasma levels of retinyl esters (29) and very high liver levels of vitamin A, due to reduced hepatic mobilization. The result is a mixed symptom pattern of hypo- and hypervitaminosis A (30, 31). Excessive hepatic accumulation of vitamin A causes liver enlargement, hepatic fibrosis, portal hypertension, splenomegaly, cirrhosis, palmar erythema, spider angiomas, and ascites. Fatigue and pruritus are the major symptoms (32, 33). Vitamin A hepatotoxicity is associated histologically with the accumulation of perisinusoidal lipocytes, associated fibrosis, obstruction of sinusoids and terminal venules, sclerosis of central veins, atrophy of adjacent hepatocytes, and an increase in basement membrane-like material and collagen within the perisinusoidal space in association with lipid-filled Ito cells (32, 34). The hepatic Ito cell is a key component in the pathogenesis of hepatic fibrosis, which occurs in both infective hepatitis and hypervitaminosis A. These vitamin A-rich stellate cells are thought to be the primary source of the extracellular matrix deposition in liver fibrosis and are also a source for the apoptotic mediators involved in fibrosis (35). During hepatic fibrosis, the vitamin A-containing stellate cells transform into myofibroblastic cells and lose their intracellular droplets of retinyl esters, the storage form of vitamin A. Recent evidence suggests that the loss of retinyl esters is associated with increased RA formation, which in turn facilitates TGF-β-mediated liver fibrogenesis (36).

Table II. Laboratory Findings in Hypervitaminosis A[a]

Laboratory tests	Hypervitaminosis A
Alkaline phosphatase	Elevated
γ-glutamyltransferase	Variable
Alanine aminotransferase	Elevated
Aspartate aminotransferase	Elevated
Bilirubin	Elevated
Prothrombin time	Elevated
Hyperlipidemia/hypercholesterolemia	Present
Retinol	Variable
Retinyl esters	Elevated
AMA	Unknown
IgM	Elevated
Hypercalcemia	Yes

[a] References: 20, 22, 54.

Pathogenesis of Liver Damage in HBV Infection

It is conjectured that the mechanism of HBV activation and the pathogenesis both of liver damage and graft failure are intimately connected to retinoid metabolism in that the HBV genome interacts with and becomes genetically coupled to RARs within the nuclei of hepatocytes. On this hypothesis, HBV infection causes hepatotoxicity by genetic coupling with RARs.

A link between viral infections and RAR expression has been reported in a number of studies. Human HBV mainly infects hepatocytes, and DNA transfection methods have shown that viral gene expression is regulated by promoter/enhancer elements. HBV contains two enhancer elements. Enhancer element I has been studied in detail at the DNA-protein level. Genetic analysis has shown that one element that plays a key role in the regulation of enhancer function is RXR-α. Using only the DNA-binding domain of the liver-specific RXR-α expressed in *E. coli*, Huan and Siddiqui (37, 38) demonstrated binding of RXR-α to the putative RAR response element (RARE) in the HBV enhancer, thereby implicating RA and its receptor in the regulation of HBV gene expression and in disease pathogenesis. Garcia *et al.* (39) have defined a novel region in HBV enhancer I, termed the GB element, the nucleotide sequence of which is similar to sequences of the DNA-binding sites for members of the steroid receptor superfamily. Among these proteins, RXR binds to the GB element *in vitro* and transactivates promoter expression via the GB element *in vivo* in response to RA. Using human hepatoma Hep3B/C16 cells as a model to examine the effect of all-trans RA on the gene expression of hepatitis B surface antigen (HBsAg), Hsu *et al.* (40) found that exposure to RA increased the level of HbsAg after 24 hr and reached a maximum at 48 hr. HBsAg expression was severely suppressed compared with the control cells after long-term (120 hr) RA treatment. This biphasic regulation of HBsAg production by RA was paralleled by the changes of HBsAg mRNA. Exposure of the cells to RA for only 8 hr was sufficient to show that up- and downregulation of the HBsAg gene occurred 2 and 5 days later.

Additionally, a DR1 hormone response element was found to be activated by peroxisome proliferator-activated receptors as heterodimers with RXR to synthesize pregenomic RNA (41). The conclusion from these studies was that HBV seemed likely to regulate its expression and replication in part via this DR1 hormone response element. Molecular studies thus provide evidence of the potential role of retinoids in the regulation of HBV replication. If retinoids and their receptors are important for HBV replication, it can be hypothesized that appropriate antagonists to nuclear receptors might be useful in the effective treatment of HBV infection.

Another connection between HBV and liver pathogenesis lies in the epidemiologic association between virus infection and hepatocellular carcinoma (42, 43). RARs play an important role in cell proliferation, and integration of a portion of the HBV genome near the site of an RAR was suggested as a potential mechanism whereby HBV might contribute to the development of hepatocellular carcinoma (44). RA might therefore play a role in HBV-associated hepatocellular carcinoma. Whether HBV causes cancer directly or simply causes the proliferation of hepatocytes, thus promoting the accumulation of genetic mutations that might lead to hepatocellular carcinoma, is uncertain (6). Several investigators have championed the role of HBV in the development of this cancer (45, 46).

Graft Failure

These data suggest that endogenous retinoid receptor expression could play a role in HBV-associated cholestasis and liver damage. As for the problem of graft failure following liver transplantation in patients with pre-existing HBV infection, our suggestion is that since graft recipients still have HBV infection, the cycle is repeated as before; that is, the virus continues to utilize retinoid receptors in the new liver, thereby contributing to cholestasis, fibrosis, and ultimately graft failure (Fig. 2). Transplantation thus exposes the grafted liver to the continuing presence of HBV infection, which re-initiates the destructive process. In contrast, transplantation as a sequel to alcoholic liver disease has a high rate of success compared with that following HBV infection, perhaps because the graft is not exposed to the virus and its presumed use of the retinoid pathways. Immunocompromised patients, such as renal transplant patients receiving immunosuppressive therapy, can be infected with hepatitis B. These patients can develop liver failure in spite of a diminished immune response (47). Since the normal immune-mediated liver destruction would be absent, it would be of interest to determine if the liver damage observed in these patients is associated with retinoid toxicity.

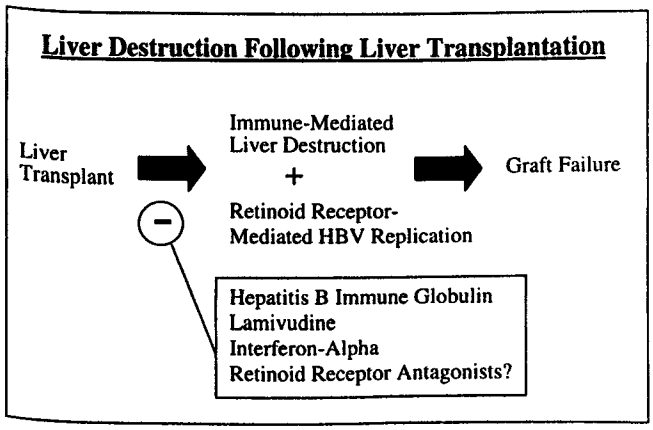

Figure 2. Our model of HBV-induced liver destruction includes retinoid receptor-mediated HBV replication and consequent immune-mediated effects. Included above are various treatment modalities that may slow or stop liver damage (denoted by the negative sign). Clinical studies will be required to test the hypothesis that retinoid receptor antagonists might be used in conjunction with current treatment options to extend liver graft survival.

Possible Use of Retinoid Receptor Antagonists in the Treatment of HBV Infection

At one time, the only treatment available to chronic HBV carriers was interferon-α, which provided variable results (11). Typical response rates in those who acquired HBV as adults ranged from 25%- to 50%, and patients infected at birth had lower response rates (10). Whereas a select population of chronic carriers are still amenable to interferon-α therapy, current therapeutic approaches include the use of nucleoside analogs such as lamivudine. Lamivudine reduces HBV DNA replication in nearly all treated patients with chronic HBV infection (48). Two studies have shown that lamivudine reverses FCH in renal transplant patients (49, 50). An adverse effect of lamivudine therapy after extended use is the rise of drug-resistant mutants that alter the DNA polymerase by nucleotide substitution (51). Treatment of chronic carriers who have undergone liver transplantation with lamivudine, along with hepatitis B immune globulin, may prevent graft infection with HBV. This regimen may be effective in treating post-transplant recurrent HBV infection (13), although the development of drug-resistant virus mutants remains a problem for all patients treated with lamivudine.

A review of the literature revealed a paucity of retinoid receptor antagonists that have been tested clinically, although there are compounds that might be good candidates (52, 53). It may be possible to utilize retinoid receptor antagonists to synergize with the actions of lamivudine and possibly prevent the rise of lamivudine-resistant virus mutants. In addition, retinoid receptor antagonists might be used to inhibit HBV replication in patients who have developed lamivudine drug-resistant virus. Retinoid receptor antagonists may also reduce the risk of hepatocellular carcinoma in chronic carriers.

Conclusions

Molecular studies have shown the potential importance of retinoids to HBV replication. Retinoid antagonists could be useful in preventing or treating the recurrence of HBV infection in liver transplant patients. The potential role of retinoid antagonists could also be investigated in preventing the development of drug-resistant HBV in chronically infected individuals. The contribution of retinoids and retinoid receptors to the pathogenesis of HBV is an area ripe for scientific study.

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