HBV-Associated Cryoglobulinemic Vasculitis: Remission after Antiviral Therapy with Entecavir

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Key Words
Hepatitis B virus • Cryoglobulinemia • Vasculitis • Entecavir • Antiviral therapy

Abstract
Background/Aims: Cryoglobulinemic vasculitis remains an uncommon complication of hepatitis B virus infection. Methods: We report the case of a 40-years old female Chinese patient with chronic hepatitis B developing cryoglobulinemic vasculitis with multiple organ involvement (liver, kidney, and skin) coupled with weakness, arthralgias, haemolytic anaemia, and autoimmune thyroiditis. She received entecavir mono-therapy at dose adjusted for estimated glomerular filtration rate. Results: Within five months of entecavir treatment, hepatitis B viraemia decreased below the limit of detection with normal serum amino-transferase levels, HBeAg clearance occurred, vasculitis regressed with disappearance of purpura and ascites; in addition, renal function normalized and nephritic syndrome remitted. After a five-year follow-up, the patient is asymptomatic with intact kidney function, proteinuria in the normal range, and normal liver biochemistry, despite the antiviral treatment was withdrawn and the patient remained HBsAg positive. Conclusions: This is the second case of hepatitis B virus-related cryoglobulinemic vasculitis successfully treated with entecavir suggesting that effective antiviral therapy may counteract both the hepatic and extra-hepatic manifestations of infection by hepatitis B virus.
Introduction

It has been calculated that approximately 350 million people are chronically infected with the hepatitis B virus (HBV) all over the world [1]. The clinical manifestations of HBV range from acute or fulminant hepatitis to various forms of chronic infection, including an inactive carrier state, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [2]. In addition, as many as 20% of patients with HBsAg experience extra-hepatic manifestations including dermatitis, poly-arthritis and arthritis, pulmonary disease, aplastic anemia, glomerulonephritis, and vasculitis [2]. The mechanism of these extra-hepatic manifestations is thought to be linked to immune complex disease but their pathogenesis is poorly clarified. Reported cases of HBV-related cryoglobulinemic vasculitis are rare, the appropriate treatment is still unknown as no pertinent guidelines have been issued to date [1, 3]. Immunosuppressive therapies, by virtue of their mechanisms of action, have the potential to have a permissive effect on HBV replication resulting in an accelerated liver injury and worsening of extra-hepatic complications including vasculitis. On the other hand, evidence on antiviral treatment with nucleos(t)ide analogues (NUCs) is extremely limited [4-11].

We report on a 40-years old female Chinese patient who experienced HBV reactivation and developed cryoglobulinemic vasculitis with purpuric skin rash, nephritic syndrome and ascites. Other manifestations included autoimmune thyroiditis, and hemolytic anemia. She was successfully treated with entecavir (ETV) mono-therapy leading to full and rapid remission of liver, skin, and kidney abnormalities. In addition, a review on the current approaches for the treatment of HBV-related cryoglobulinemic vasculitis has been made.

Case Study

A 40 year-old female Chinese was admitted (September 2008) with two week’s duration of fatigue and abdominal pain. She had a known history of chronic hepatitis B (hepatitis B surface antigen (HBsAg) positive and hepatitis B envelope antigen (HBeAg) positive) infection with high serum HBV viral load (HBV DNA, >8 log_{10} IU/mL) and normal alanine amino-transferase (ALT) levels suggesting an immunotolerance condition. There were not co-morbidities, co-infection with hepatitis C virus (HCV), delta virus (HDV), or human immunodeficiency (HIV) virus. History of alcohol or drug consumption, exposure to illicit drugs, hazardous sexual habits, travel to exotic destinations or parenteral risks were excluded after careful questioning. Familiar medical history was positive for liver disease of unknown origin, arterial hypertension, and neoplastic disorders. On physical examination, she presented arterial hypertension (190/100 mmHg), mild fever (37.3°C), and tenderness of the ankles. Abdominal ultrasonography showed abundant ascites, mild hepatomegaly, and increased echogenicity of kidneys. Laboratory data at presentation were as follows: hemoglobin, 11.2 g/dL; leucocytes, 21,200/mm³; platelets, 125,000/mm³; C-reactive protein, 3.36 mg/dL; ALT, 178 IU/L; creatinine, 1.77 mg/dL; blood urea nitrogen (BUN), 90 g/L; total bilirubin, 1.40 mg/dL; albumin, 2.0 gr/dL; cholinesterasis, 2799 IU/L; and international normalized ratio (INR), 1.24 (Table 1). The estimated glomerular filtration rate (eGFR) according to the Modification of Diet in Renal Disease (MDRD) study equation was 33.8 mL/min. Proteinuria 3+, and hematuria 4+ were observed by urinalysis. Test results for HBsAg, HBeAg and hepatitis B core antibody (HBCAb) IgG were positive, whereas test results for antibodies against HBsAg (HBsAb), hepatitis B envelope antibody (HBeAb) and HBCAb IgM were negative. Serum HBV DNA, as measured by real-time PCR, was 12,238,800 IU/mL. Serologic markers were negative for IgM antibody to hepatitis A virus, Epstein-Barr virus, cytomegalovirus, herpes simplex virus, herpes zoster virus and toxoplasma, as well as the antibody to HCV, delta virus (IgG/IgM) and HIV (all tested by standard immunoenzymatic methods). Autoimmune hepatitis was also excluded by a negative test for serum tissue auto-antibodies as were Wilson’s disease (ceruloplasmin, serum and urine copper), α1-antitrypsin deficiency and celiac disease.
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Kidney function deteriorated over the first week after admission, serum creatinine up to 3.46 mg/dL (eGFR by MDRD equation, 15 mL/min) (Table 1) with peripheral edema and purpura over the lower extremities. Repeat urine sediment, analyzed by phase-contrast microscopy, showed severe microscopic haematuria (80-100 erythrocytes/microscopic field), numerous dysmorphic erythrocytes and casts (granular and red cell casts); 24-hour protein excretion ranging between 1 and 2.5 gr (non-nephrotic proteinuria). Kidney biopsy was not performed due to long bleeding time. Arterial hypertension required treatment with various anti-hypertensive medications including angiotensin converting enzyme inhibitors (ACE-inhibitors, ACEi) and angiotensin-receptor blockers (ARBs).

Hemolytic anemia was diagnosed and several units of packed red blood cells were administered. Moreover, plasma thyroid stimulating hormone (TSH) levels were increased (7 μUI/mL) with normal free T₃ (FT₃), and free T₄ (FT₄) but with positive status for anti-thyroperoxidase antibody (TPO-Ab) and anti-thyroglobulin antibody (Tg-Ab), being 554 (<4) and 99 (<10) IU/mL, respectively; this suggested an autoimmune thyroiditis requiring levothiroxine supplements. Serum complement fractions were reduced- C₃, 43 mg/dL (79-152) and C₄, 5 mg/dL (16-38), respectively. Serum cryoglobulins were positive (cryocrit, 3%). Anti-phospholipid antibodies, Bence Jones protein as well as antinuclear and anti-neutrophil cytoplasmic antibodies were negative. Auto-antibodies directed against Ro/SSA and La/SSB autoantigens were not found. Rheumatoid factor tested positive whereas serum protein electrophoresis and serum immunoglobulins were normal. The search for neoplastic cells in the ascitic fluid was negative. Small ischemic abnormalities of the brain were observed by magnetic resonance; colonoscopy revealed mucosal hyperemia with small hemorrhagic areas throughout the left colon, non-specific chronic colitis was found by biopsy. GERD (gastro-esophageal reflux disease) was diagnosed by esophago-gastro-duodenoscopy; chest X-ray excluded inflammatory or neoplastic changes.

We excluded potential causes of mixed cryoglobulinemia such as neoplasias or autoimmune disorders, and HBV-associated cryoglobulinemic vasculitis was diagnosed. Antiviral therapy with entecavir, 0.25 mg every 72 hours (due to reduced eGFR) was

| Table 1. Blood chemistries and viral markers at presentation and over follow-up |
|------------------|------------------|------------------|
| Creatinine (0.5-1.2; mg/dL) | 3.46 | 1.34 |
| BUN (8-20; mg/dL) | 110 | 54 |
| eGFR by MDRD, mL/min | 15 | 65 |
| ALT (5-31; IU/L) | 278 | 48 |
| γ-GT (5-36, IU/L) | 54 | 25 |
| Alkaline phosphatase (35-104, IU/L) | 164 | 123 |
| Cholinesterase (5,300-12,900, IU/L) | 2799 | 2929 |
| Total bilirubin (0.2-1.1, mg/dL) | 1.8 | 0.80 |
| Direct bilirubin (0-0.3, mg/dL) | 1.0 | 0.30 |
| Hb (12-16, g/dL) | 10 | 12.5 |
| Serum albumin (3.4-4.8, g/dL) | 2.0 | 3.1 |
| Prothrombin time (0.88-1.16) | 1.24 | 1.06 |
| Partial thromboplastin time (0.85-1.18) | 1.12 | 1.10 |
| Proteinuria (mg/24 hours) | 2540 | 810 |
| HBsAg/anti-HBs | Pos/Neg | Pos/Neg |
| HBeAg/anti-HBe | Pos/Neg | Pos/Neg |
| Anti-HBV IgM core | Neg | Neg |
| HBV DNA (IU/mL) | 12,238,000 | 101,900 |

Abbreviations: BUN, blood urea nitrogen; ALT, alanine aminotransferase; γ-GT, glutamyl transpeptidase; HBV DNA, viralemia of hepatitis B virus
initiated. Soon after the beginning of entecavir therapy, AST, ALT, and HBV DNA values lowered; kidney function improved with non-nephrotic proteinuria (Figure 1). Her general health improved with resolution of edema, ascites, and arthralgia; the vasculitic skin rash gradually cleared even if purpuric rashes over the lower extremities recurred several times. At discharge from the Hospital (November 2008), serum creatinine was 1.34 mg/dL, BUN 54 g/L, and 24-hour proteinuria 810 mg. The eGFR by MDRD equation improved significantly (65 mL/min), the ETV dose was increased to 0.5 mg once daily. Other pertinent biochemistries were: ALT 25 IU/L, total bilirubin 0.80 mg/dL, γ-GT (glutamyl-transpeptidase) 25 IU/L, cholinesterases 2929 IU/L, C3 46 mg/dL, C4 5 mg/dL, serum albumin 3.1 g/dL, and serum HBV DNA 101,900 IU/mL. Urine sediment revealed persistent microscopic haematuria (80-100 erythrocytes/microscopic field) and numerous casts (granular and red cell casts). Her medications at that time included ETV, calcium antagonists, ACEi, beta blockers, ARBs, furosemide, omeprazole, and levothyroxine.

One month after discharge (December 2008), liver biochemistry was as follows: ALT 36 IU/L, cholinesteras 5135 IU/L, γ-GT 27 IU/L, and HBV DNA 8,900 IU/mL. Renal function became normal (serum creatinine 0.99 mg/dL, BUN 30 mg/dL and eGFR 66 mL/min), serum albumin 4 g/L, and protein excretion 211 mg/24 hours (<150 mg/day). Serum cryoglobulins disappeared and levels of complement returned within the normal range. Urine sediment was consistent with microscopic mild haematuria (10-15 erythrocytes/microscopic field), red cell casts being absent. Five months after starting ETV (February 2009), while the patient maintained normal AST and ALT levels, for the first time she achieved undetectable serum HBV DNA (<12 IU/mL) and developed anti-HBe antibody (with HBeAg positive status). At that time, serum creatinine was 0.77 mg/dL, and proteinuria 84 mg/24 hours. In July 2009 (10 months after starting ETV), the patient became definitively HBeAg negative with anti-HBe positivity; antiviral therapy was discontinued in September 2011.

At the last observation in December 2013 (five years after the referral to our Hospital and 27 months after ETV discontinuation), the patient remained asymptomatic with appropriate control of blood pressure, kidney function was normal (serum creatinine, 0.58 mg/dL; eGFR 116 mL/min) and proteinuria (40 mg daily) in the normal range. She showed normal amino-transferase values, low serum HBV DNA levels (409 IU/mL) and confirmed anti-HBe sero-positive status (a condition suggesting an inactive HBV carrier). Repeat urine sediment tested positive for mild microscopic haematuria (1-4 erythrocyte/microscopic field). Abdominal ultrasonography showed normal liver and kidneys whereas transient elastography (Fibroscan) gave normal liver stiffness (3.7 kPa). The patient is currently
asymptomatic, her medications include: bisoprolol 2.5 mg once daily, levothyroxine 75 mg day, and enalapril 20 mg daily.

**Review of the literature**

There is a paucity of data on the treatment of HBV-related cryoglobulinemic vasculitis; this is probably due to the uncommon occurrence of HBV-associated cryoglobulinemic vasculitis. Also, the implementation of universal HBV vaccination programs is producing a significant decrease in the horizontal transmission of HBV [12]; an additional decline of the frequency of HBV-positive cryoglobulinemia vasculitis in the developed world is expected in the near future.

A better understanding of the mechanisms of disease has provided the opportunity to control HBV-associated mixed cryoglobulinemia (MC) using targeted approaches: 1) antiviral therapy, according to the hypothesis that the underlying infection instigates the synthesis of immune complexes and the ensuing vasculitis; 2) B–cell depletion therapy targeting B cells that produce cryoglobulins; and 3) immunosuppressive therapy targeting inflammatory cells present in vasculitic changes. Similarly to HCV-positive MC [13], some evidence in the scientific literature exists on the treatment of HBV-induced cryoglobulinemic vasculitis with targeted strategies. Antiviral treatment, mostly based on mono-therapy with NUCs such as lamivudine [4-6, 9], adefovir dipivoxil [7], entecavir [8], or telbivudine [10] has given encouraging results in terms of viral clearance and clinical remission in HBV-associated cryoglobulinemic vasculitis (Table 2). Moreover, NUCs have been also administered with some benefit for HBV-associated nephropathy [14-15]. Another patient with renal insufficiency received ETV for acute hepatitis and severe myopathy at lower limbs, probably related to HBV-associated vasculitis; the outcome being excellent [16].
A positive effect of interferon-α (IFN-α) on the clinical, serological, and virological parameters has been reported in one HBsAg positive patient with symptomatic MC (recurrent purpura, mild sensory peripheral neuropathy, and hepatitis). Rapid improvement of purpura, liver enzymes, cryocrit, and clearance of HBV DNA from serum were observed after a 4 week treatment period. However, the progressive worsening of peripheral neuropathy (impairment in walking due to motor neuropathy) occurred during antiviral therapy [17]. A clear reduction of neurological symptoms with complete recovery of the gait was observed after discontinuation of IFN-α. Treatment with interferon-alpha was found to be effective in symptomatic mixed cryoglobulinemia even in the presence of HBe-minus HBV mutants [18].

An additional option for the treatment of HBV-associated cryoglobulinemic vasculitis should be rituximab (RTX), that is a chimeric antibody that binds to the B-cell surface antigen CD20 and results in rapid depletion of circulating and tissue B cells. RTX interferes with synthesis of cryoglobulins and monoclonal IgM. The case of a Caucasian male who was refractory to immunosuppressive and antiviral therapy and received RTX therapy, was recently reported [19]; B-cell depletion was safe and effective in inducing a complete remission of the multisystem disease (leukocytoclastic vasculitis, arthralgias, nephrotic syndrome, Raynaud’s phenomenon).

Non specific immunosuppressive agents such as combined therapy with corticosteroids and immunosuppressive agents (cyclophosphamide and azathioprine) have been tried in patients with HBV-positive cryoglobulinemic vasculitis and life-threatening disease complications (i.e., progressive kidney insufficiency, severe neuropathy, or extensive skin disease) [20]. Immunosuppressive therapy has been made while awaiting the generally slow response to antiviral treatment. Plasma exchange and a low antigen content diet to reduce cryoglobulins and their inflammatory effects have also been given with some benefit [20].

**Discussion**

To our knowledge this is the second case of hepatitis B virus-related cryoglobulinemic vasculitis successfully treated with entecavir suggesting that effective antiviral therapy may counteract both the hepatic and extra-hepatic manifestations of infection by hepatitis B virus. Our patient shows interesting findings including the multiple organ involvement (skin, liver, and kidney), the beneficial effect of mono-therapy with ETV on clinical, virologic, and serologic parameters, and the role of HBV infection in cryoglobulinemia. She showed high HBV viremia with concomitant high ALT levels indicating an immunoclearance phase of HBV infection. The severity of hepatic and extra-hepatic manifestations prompted us to start immediately an antiviral therapy, leading to a promptly reduction of serum HBV DNA and ALT levels, and, most important, to an impressive improvement of kidney function and clinical conditions. Purpura disappeared even if some recurrence of skin rashes were observed over a few weeks after HBV DNA clearance. After a five-year follow-up, the patient is asymptomatic with intact kidney function, appropriate control of blood pressure, liver biochemistries and proteinuria in the normal range despite the antiviral treatment was withdrawn and the patient remained HBsAg positive.

The relationship between HBV and cryoglobulinemia is controversial, Levo et al. [21] were the first authors to suggest the association between HBV and cryoglobulinemia but later studies offered conflicting results [22-23]. It is now established that HBV is able to induce cryoglobulinemia although infrequently. After the identification of hepatitis C virus, HCV has been recognized as the cause of 80%-90% of mixed cryoglobulinemia (MC) [13]; according to a large series of patients with mixed cryoglobulinemia, anti-HCV seropositive status was observed in 92% (155 of 168) of cases, whereas hepatitis B surface antigen was detected in 9% (15/168) [20].
Cryoglobulinemia is a pathologic condition in which the blood contains immunoglobulins having the property of reversible precipitation from human serum cooled to 4°C. Cryoglobulinemia vasculitis is a systemic vasculitis that involves mostly small and, less frequently, medium-sized arteries and veins. The antigenic stimulus maintained by HBV determines the polyclonal and later oligo-monoclonal expansion of B-cells with the appearance of cryoglobulins and rheumatoid factor. Immune complexes formed by HBV, polyclonal IgG, and monoclonal IgM (provided with rheumatoid factor activity) are deposited on endothelial surfaces, causing vascular inflammation. MC characteristically represents a form of immune complex vasculitis; intravascular cryoglobulin precipitation being instigated by cold temperature, and may involve primarily the skin, peripheral nerves, and kidney [24].

Clinical practice guidelines for the treatment of HBV-associated cryoglobulinemic vasculitis have not been issued to date [1, 3]. We suggest to start antiviral therapy with NUCs for patients with HBV-induced cryoglobulinemic vasculitis whose disease severity and activity is mild to moderate. For patients having severe disease (defined as progressive motor neuropathy, or rapid kidney insufficiency, or skin ulcers), a treatment with RTX and/or plasma exchange and/or conventional immunosuppressive agents is recommended, concomitantly with therapy by NUCs as the risk of further HBV reactivation, leading to death in some cases, has been reported (Figure 2) [25-27]. Fulminant hepatitis with death due to HCV reactivation in renal transplant recipients who had received RTX therapy has been also noted [28]. Pre-emptive use of entecavir has provided successful management of HBV reactivation but moderate hepatic flares can still occur [29].

Several questions concerning the treatment of HBV-positive cryoglobulinemic vasculitis remain unclear as the time to initiate antiviral drugs, the role of kidney/liver biopsy before antiviral therapy, and the appropriate duration of antiviral therapy, among others. An acceptable goal of HBV therapy should be viral suppression instead of eradication, although this may require long-term treatment in chronic carriers. This is not a trivial point as long-term therapy with NUCs may be hampered by patient compliance, financial burden, resistance mutations, and potential toxicity. On the other hand, in patients with mild liver disease, as in our case study, antiviral therapy may be discontinued after persistent HBeAg seroconversion, as recommended by international guidelines [1].
Conclusion

In conclusion, this case study gives emphasis to the efficacy and safety of mono-therapy by ETV for HBV-associated cryoglobulinemic vasculitis. Of note, all published treatment data stem from anecdotal reports; hence, we call for well-designed randomized controlled trials of antiviral therapy towards HBV-induced cryoglobulinemic vasculitis.

Disclosure Statement

Mauro Viganò: Speaking and Teaching: Roche, Gilead Sciences, BMS.
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