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REVIEW

Emerging role of dual antiplatelet therapy in the prevention of hepatitis B virus-associated hepatocellular carcinoma

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Abstract: Platelets, the chief effectors of vascular homeostasis, have been identified as important players in the pathogenesis of both acute and chronic liver disease in preclinical models of hepatitis B viral infection. Platelets are thought to promote the accumulation of virus-specific T-cells into the liver parenchyma. Importantly, the inhibition of platelet activation by clinically relevant doses of aspirin and clopidogrel was able to reduce immune-mediated necroinflammatory liver disease, extracellular matrix deposition, and hepatocellular carcinoma development; the same treatment was able to improve overall survival. These results strongly support the design of clinical trials aiming to define the potential of antiplatelet therapy in the prevention of hepatitis B virus-associated hepatocellular carcinoma.

Keywords: platelets, hepatocellular carcinoma, hepatitis B virus

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cause of death from cancer worldwide.[1] Liver infection with the hepatitis B virus (HBV) is a major etiological factor for HCC; this type of liver infection is responsible for over 50% of the reported cases of HCC.[1] Among the described mechanisms by which HBV causes HCC, two are thought to be prominent: viral factors, such as the expression of viral gene products with procarcinogenic potential and insertional mutagenesis; and host factors linked to the antiviral immune response.[2] In particular, HBV-associated chronic hepatitis, characterized by a dysfunctional virus-specific $CD8^+$ T-cell response that is unable to clear the virus from the infected hepatocytes, appears to play a major role in promoting continuous cycles of hepatocellular death and compensatory liver cell proliferation in the context of inflammatory mutagens that, over time, are thought to promote the multiple genetic alterations associated with HCC.[3]

Overview of current treatment options and patient outcomes

Currently, several therapeutic approaches, mainly dictated by the stage of tumor progression, are available for the treatment of HCC.[4–6] Apart from very early-stage HCC, for which surgical resection or liver transplantation represent a curative option, treatment and prognosis is poor for intermediate- and advanced-stage HCC patients, who represent more than 60% of those diagnosed with HCC.[6,7] For cases in which surgical resection and liver transplantation are not possible, local ablation using radiofrequency or percutaneous ethanol injection and transarterial chemoembolization have become

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the most frequently used treatments, although they are often associated with high recurrence rates.[6–9] Furthermore, other ablative therapies such as microwave therapy, cryoablation,[6–9] and proton beam radiotherapy[10] have proved to be somewhat effective in small-scale clinical trials and are valuable alternatives that need additional clinical evaluation. Finally, individuals with advanced-stage HCC may benefit from the multikinase inhibitor, sorafenib, although this has an improvement of median survival of only 7–8 weeks in spite of its high cost.[11–13]

Given the poor prognosis and the paucity of curative treatments for HCC, new strategies aimed to prevent the long-term consequences of chronic hepatitis are highly encouraged. With recent findings from a transgenic mouse model of chronic HBV, researchers were able to demonstrate the long-term use of dual antiplatelet therapy as a preventive strategy that is able to reduce the occurrence of HCC development.[14]

The role of platelets and platelet-derived molecules in HCC development

By using different mouse models of acute viral hepatitis, our group showed that virus-specific CTLs are key triggers for hepatic necroinflammatory liver disease during the course of HBV infection.[3] In this process, platelets play an instrumental role. We illustrated this by taking advantage of two mouse models of self-limited viral hepatitis: HBV-replicating mice injected with HBV-specific CTLs; and mice infected with a hepatotropic adenovirus. Through these models, we showed that platelets can be detected at the site of liver damage, and that their depletion ameliorates liver disease by reducing the frequency and amount of virus-specific and virus-nonspecific inflammatory infiltrate recruited into the liver parenchyma.[15,16]

Importantly, we found that inhibitors of platelet activation (such as aspirin that blocks thromboxane A_2 production by irreversibly acetylating cyclooxygenase 1 enzyme, and clopidogrel that inhibits the ADP P2Y purinoreceptor 12), were able to modulate the platelet-dependent intrahepatic accumulation of virus-specific CTL.

Since the oral administration of aspirin and clopidogrel is used in clinical settings for the treatment of patients with a high risk of thrombosis, we decided to evaluate the effects of the sustained inhibition of these two platelet activation pathways on the severity of chronic liver damage and HCC development in an established mouse model of immune-mediated chronic hepatitis B.[17] After the induction of acute hepatitis and until the end of the study, mice were treated with doses of aspirin and clopidogrel that are proportionate to the doses used in patients. These mice showed a significant reduction in the accumulation of virus-specific CTLs and of virus-nonspecific inflammatory cells within the liver; these mice also showed a consequential reduction of liver disease.[14] Additionally, these mice displayed reductions in hepatocyte compensatory proliferation, collagen deposition (fibrosis), liver cell transformation, and HCC development. Importantly, treated animals showed a greater improvement in overall survival compared to the untreated mice.[14] These results indicate that platelets not only influence the intrahepatic accumulation of HBV-specific CTLs during acute hepatitis, but are able to influence conditions during the chronic stages of the disease by modulating certain inflammatory reactions. These reactions are generated by the antiviral immune response against infected hepatocytes that promote HCC transformation during chronic HBV infection.[3,18–20]

Different possible mechanisms by which platelets could influence the recruitment of virus-specific CTLs have been proposed. Upon activation, platelets can express on their plasma membranes CD40 ligand, a molecule that is able to modulate different aspects of innate and adaptive immunity to contribute to the expansion and activity of CTLs that result in the accumulation of these cells at sites of viral replication.[21–23] Furthermore, upon activation, platelet granule content is rapidly exocytosed to boost hemostasis, inflammation, and immune responses. Among these molecules, P-selectin and P-selectin glycoprotein ligand-1 have been known to play roles in the interactions between platelets, different subsets of leukocytes and the endothelial wall. Other molecules, such as platelet factor 4, are able to activate and recruit cells at sites of inflammation.[24] Furthermore, during chronic viral hepatitis and HCC development, other platelet-derived vasoactive molecules, such as serotonin, may play a role in the modulation of sinusoidal circulation, altering virus-specific CTL recirculation into the liver, ultimately contributing to liver damage.[25,26]

Mechanistically, at the dose used in our study, aspirin treatment alone inhibits the release of serotonin and other small molecules from δ-granules with no effect on the release of a wide array of immunologically relevant proteins and peptides stored in the α-granules that are instrumental in the functional cross-talk between platelets and leukocytes.[22,24] Conversely, clopidogrel has been shown to downregulate the expression of these inflammatory molecules (P-selectin and CD40L); this process is able to inhibit a number of platelet–leukocyte interactions that link

vascular injury to inflammatory reactions.[27] In this scenario, the inhibition of different platelet activation pathways that is achieved by aspirin- and clopidogrel-combined therapy may ameliorate the course of immune-mediated chronic hepatitis and HCC progression through the distinct pharmacological effects of the two different drugs. The synergistic effect of aspirin and clopidogrel may reduce platelet influence in a complex pathogenic mechanism in which multiple pathways contribute to the intrahepatic accumulation of virus-specific CTLs.[14,19,20]

Critical analysis of the potential role of antiplatelet therapy in the clinical setting

Our preclinical study shows that inhibiting platelet activation by using two widely used antiplatelet drugs is able to ameliorate the development of HBV-induced immune-mediated HCC, delay it, or both.[14] Although the data presented in our study represent a step forward in the understanding of HBV-associated hepatocarcinogenesis, caution has to be taken before antiplatelet treatment may be applied in clinic. Since the HBV transgenic mouse model that is used does not support infection and viral replication, the outcome of combined antiplatelet therapy on these parameters should be carefully monitored.[14] The antiplatelet therapy-mediated reduction of virus-specific CTL accumulation into the liver may have negative effects on the control of HBV viremia. Simultaneous use of nucleoside and nucleotide inhibitors might be supportive in limiting virus replication in patients subjected to antiplatelet therapy. Moreover, since the transgenic mouse model used in the preclinical study lacks the expression of the whole HBV genome, there is the potential to underestimate the role of viral gene products with transforming potential in HCC development during HBV chronic infections. For these reasons, additional preclinical work using the woodchuck hepatitis virus model may give further information on viral replication, liver disease severity, and oncogenic potential in the context of combined antiplatelet therapy. Finally, the increased risk of bleeding might limit the long-term use of aspirin and clopidogrel in some patients affected by chronic liver diseases. However, the findings from a recent report suggest that patients with thrombosis may be at higher risk and, therefore, might benefit from this approach.[28] In addition, the long-term use of aspirin was recently shown to reduce the risk of HCC in patients suffering from chronic liver disease of unknown etiologies.[29] These observational results, together with our preclinical work, strongly support the basis for future clinical trials aimed at evaluating the effect of single or combined antiplatelet therapy in the prevention of HCC development in HBV-infected patients.

Highlights

- Platelets promoted the accumulation of virus-specific CTLs into the liver parenchyma in mouse models of acute and chronic hepatitis B infection.
- Long-term treatment with aspirin and clopidogrel reduced platelet-dependent accumulation of virus-specific CTLs and consequently reduced liver inflammation.
- In mouse models of immune-mediated chronic hepatitis B, continuous administration (approximately 600 days of treatment) of aspirin and clopidogrel diminished the severity of persistent liver damage and of liver fibrosis without causing significant side effects. These effects prevented HCC development and improved overall survival.
- Drugs targeting platelet functionality may present a novel preventive strategy for patients with chronic HBV infection who are at risk of developing liver cancer.

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Disclosure

The authors report no conflicts of interest in this work.

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