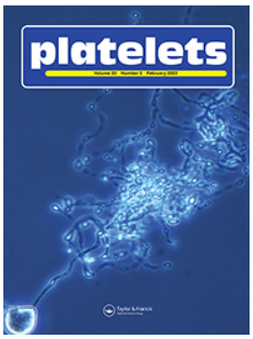

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REVIEW

Antiplatelet drugs and liver fibrosis

Pamela Czajka [1], Adam Przybyłkowski [2], Anna Nowak [1], Marek Postula [1], Marta Wolska [1], Dagmara Mirowska-Guzel [1], Anna Czlonkowska [3], & Ceren Eyileten [1]

[1]Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, Center for Preclinical Research and Technology CEPT, Warsaw, Poland, [2]Department of Gastroenterology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland, and [3]2nd Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland

Abstract

Liver fibrosis results from an imbalance between extracellular matrix formation and degradation. The background of liver fibrosis is chronic inflammation and subsequent microcirculation disturbance including microthrombosis. Platelets actively participate in liver fibrosis not only as a part of the clotting system but also by releasing granules containing important mediators. In fact, platelets may play a dual role in the pathophysiology of liver fibrosis as they are able to stimulate regeneration as well as aggravate the destruction of the liver. Recent studies revealed that antiplatelet therapy correlates with inhibition of liver fibrosis. However, liver impairment is associated with extensive coagulation disorders thus the safety of antiplatelet therapy is an area for detailed exploration. In this review, the role of platelets in liver fibrosis and accompanying hemostatic disorders are discussed. Additionally, results of animal and human studies on antiplatelet drugs in liver disorders and their potential therapeutic utility are presented.

Keywords

Acetylsalicylic acid, antiplatelet treatment, hepatocellular carcinoma, liver fibrosis, $P2Y_{12}$ inhibitors, platelets

History

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Introduction

Liver cirrhosis is the end-stage of chronic liver diseases (CLD) and causes 2.4% of deaths worldwide per year [1]. The essence of cirrhosis pathogenesis is an imbalance between the formation and degradation of the extracellular matrix (ECM) in response to chronic inflammatory reaction [2,3]. Noxious stimuli e.g. viruses, toxins or alcohol cause hepatocytes injury, induce in response chronic inflammation, excessive fibrogenesis and finally cirrhosis. Fibrotic reaction is associated with microvascular changes such as the formation of intrahepatic shunts, sinusoidal remodeling caused by extracellular matrix deposition and endothelial dysfunction. The endothelial dysfunction is characterized by decreased production of vasodilators, especially nitric oxide and increased release of vasoconstrictors e.g. thromboxane A2 (TXA2) [4,5]. Furthermore, activated liver sinusoidal endothelial cells (LSECs) secrete a number of proinflammatory chemokines and cytokines intensifying inflammation and vasoconstriction [6]. Increased stiffness and subsequent decreased permeability of liver sinusoids leads to portal hypertension and ascites. Cirrhosis is a dynamic reaction, which can progress rapidly in a few years or more slowly, during decades, depending on underlying causes. The progression is not linear, early stages characterized with 1% one-year mortality increasing dramatically to 57% in advanced stages with decompensated liver function. Very often CLD is almost asymptomatic until cirrhosis with clinical decompensation occurs [7]. Thus, the challenge of cirrhotic patients management is prevention and stabilization in the early stages to avoid progression and to delay clinical decompensation.

Platelets play an important role not only in blood clotting but also in the immune response including cell activation, angiogenesis, and healing of the injuries [8,9]. They participate in the intercellular crosstalk and regulate the function of other cells through their ability to release particular granules (cellular components of platelets) containing various mediators. Both dense (containing small molecules) and alpha (containing chemokines adhesion proteins, growth factors and coagulation factors) platelet-derived granules are released after platelet activation and can promote healing or intensifying the destruction of tissues [10,11]. The dual role of platelets is observed in CLDs. They enhance hepatocyte proliferation and regeneration of parenchyma. On the other hand, they augment liver injury by activation of endothelial cells, stimulation of leukocyte recruitment to sinusoids, aggravation of microvascular dysfunction [12,13]. Recent studies have shown that antiplatelet therapy can inhibit the progression of liver fibrosis [14–16]. However platelet contribution in liver cirrhosis remains to be elucidated. This review is aimed to integrate current understanding in the field of platelet function in liver fibrosis, to demonstrate its appearance in the clinical feature, as well as to show the most recent studies on the impact of the antiplatelet drugs on the minimization of the risk of liver fibrosis and hepatocellular carcinoma, and in conclusion to describe the possible mechanisms of their actions.

The platelets and pathogenesis of liver fibrosis

Injurious factors such as toxins and viral infections initiate liver inflammation and activate Kupffer cells (KCs) and platelets. They release PAF, a proinflammatory molecule activating platelets and mediating in leukocyte adhesion to LSECs, which contribute to microvascular dysfunction [17]. Besides, platelets and KCs produce also transforming growth factor beta (TGF-β) which activate hepatic stellate cells (HSCs-Ito cells) and promote their transformation into myofibroblasts. Activated HSCs intensively produce collagen and synthesize a tissue inhibitor of metalloproteinases, inhibiting collagen degradation [18]. Fenestrated LSECs become

Correspondence: Adam Przybyłkowski, Department of Gastroenterology and Internal Medicine, Medical University of Warsaw, Warsaw, Poland. Email: adam.przybylkowski@wum.edu.pl; czajka.pamela@gmail.com

capilarized and lose their function. Portal hypertension and metabolic dysfunction of the liver develop and potentiate further degradation of hepatocytes. KCs release TXA2 - vasoconstrictive eicosanoid stimulating leukocyte adhesion, platelet aggregation and synthesis of TNF (tumor necrosis factor) [19]. TNF is produced by hepatocytes, activated macrophages, monocytes and CD8 + T lymphocytes [20,21]. Elevated concentration of TXA2 leads to increased resistance in the portal venous system, followed by portal hypertension, while TNF maintains high intensity of inflammation. The reduction of liver blood flow aggravates the injury of hepatic cells and augments fibrosis [22].

Platelets participate in the fibrosis due to their thrombotic and inflammatory-mediating abilities. They play a pivotal role in cellular crosstalk by secretion of granules containing a number of cytokines and chemokines. Activated platelets release platelet-derived growth factor (PDGF) and TGF-β. Platelet-derived TGF-β was shown to promote liver fibrosis through initiation of profibrotic signaling in HSCs and activate collagen synthesis [23]. PDGF enhances the proliferation of activated HSCs and portal fibroblasts, and acts as a chemoattractant for inflammatory cells [24,25]. Moreover, platelets are able to activate LSECs to express chemokines as well as promote neutrophils and lymphocyte recruitment [26]. It was shown that the recruitment of leukocytes driven by platelets results in hepatic damage during systemic endotoxemia in rodent models [27]. Furthermore, platelets are able to stimulate LSECs to produce regenerative factors like vascular endothelial growth factor (VEGF) and interleukin 6 (IL-6) through the sphingosine-1-phosphate pathway. IL-6 inhibits apoptosis and stimulates hepatocyte proliferation through the STAT3 pathway [28,29]. Higher levels of IL-6 were observed in cirrhosis and hepatocellular carcinoma (HCC) [30]. VEGF maintains HSCs quiescence by stimulating LSECs differentiation in physiological conditions, but when fibrosis progresses the VEGF production is increased promoting liver angiogenesis by HSCs activation. The direct action of VEGF on HSCs and the release of TGF-β by capillarized LSECs is responsible for intensifying HSCs activation [28,29]. On the contrary, rodent studies showed that platelets suppressed HSCs activation through hepatic growth factor (HGF) secretion and the HSCs inhibition resulted in the reduction of collagen synthesis and decreased expression of TGF-β [29,31]. Moreover, platelets release thrombin, a procoagulant factor that can both activate HSCs and increase the secretion of monocyte chemoattractant protein-1(MCP1), one of the key chemokines that regulate migration and infiltration of monocytes, enhancing inflammation and fibrogenesis [32]. Activated platelets express CD40 ligand (CD154), which interacts with CD40 on endothelial cells and activates them. Activated endothelial cells express CCL2 and upregulate expression of VCAM-1 and ICAM-1, which increases leukocyte recruitment leading to aggravation of inflammation [33]. Likewise, serotonin released by platelets acts as a chemoattractant for inflammatory cells - leukocytes, granulocytes, dendritic cells and macrophages [34]. Activated platelets express on their plasmatic membrane *P*-selectin, a molecule activating neutrophils by interaction with CD24 and stimulate them to the expression of integrins responsible for other immune cell recruitment. Besides, platelets synthesize TXA2, the prostaglandin vital for the regulation of inflammation. TXA2 is a vasoconstrictor and procoagulant factor and contributes to endothelial cell activation and platelet aggregation [35]. Due to the fact that chronic inflammation as well as microcirculatory hemostasis imbalance are postulated mechanisms leading to disease progression the role of platelets seems to be important. Thus, treatment inhibiting platelet activation was studied as a potential factor inhibiting liver fibrosis progression. However, it is worth noting that liver cirrhosis is associated with complex hemostasis disorders, raising doubts as to the use of antiplatelet therapy in clinical practice. Enhanced platelet aggregation and vasoconstriction form ideal conditions for a hypercoagulability state. On the other side, the most common cirrhosis-related defect of hemostasis is thrombocytopenia exacerbated by a reduced number of circulating platelets due to increased splenic sequestration and platelet consumption accompanied by decreased synthesis and intensified degradation of thrombopoietin (TPO) [36,37]. Besides thrombocytopenia, the qualitative platelet defects are observed, such as reduction of TXA2 synthesis, decreased ATP and serotonin concentration in dense granules, impairment of response on profibrotic agents and increase of platelets inhibitors production [38]. A few studies reported the upregulation of the factors promoting platelet activation in liver fibrosis e.g. an adhesive protein von Willebrand factor (vWF), however, it is thought to be the compensation mechanism subsequent to platelet count reduction [39–42]. Moreover, the liver synthesizes factors, which are crucial in the coagulation cascade, and fibrinolytic system (Figure 1). Deterioration of liver function results in impairment of the coagulation system at each level. Decreased concentration of fibrinogen and plasma coagulation proteins, especially factor V and factor VII due to the shortest half-live times are sensitive markers of liver synthetic capacity impairment. Similarly, the concentration of the anticoagulant protein C is lower in patients with liver failure whereas the concentration of factor VIII may increase [43,44]. The imbalance between procoagulant and anticoagulant factors increase bleeding risk as well as thrombotic risk. The overall effect is difficult to predict in a particular patient and remains a clinical challenge.

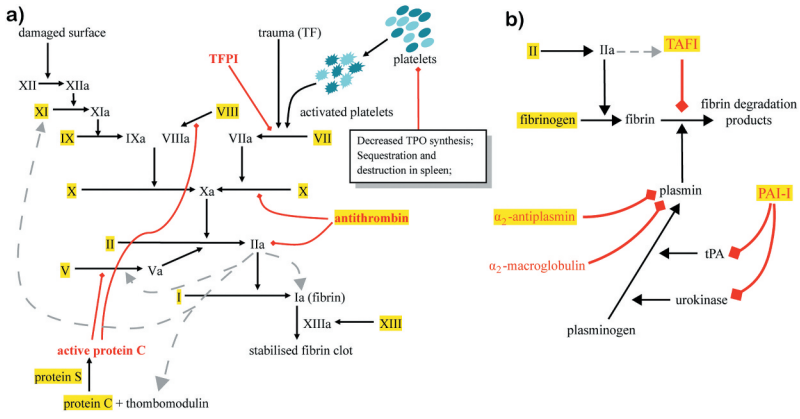

Figure 1. **Coagulation cascade and fibrinolytic system. a) The coagulation cascade**. Concentration and activity of all the factors synthesized in the liver (yellow highlighted) may be dysregulated in liver cirrhosis, resulting in increased bleeding risk as well as an increased thrombotic risk. Moreover, chronic liver disease is also characterized by primary hemostasis disorder, due to the decreased levels of thrombopoietin and intensified platelets sequestration in the spleen. **b) The fibrinolytic system**. Concentration and activity of all the factors synthesized in the liver (yellow highlighted) may be dysregulated in liver cirrhosis. Abbreviations: TF – tissue factor, TFPI – tissue factor pathway inhibitor, TPO – thrombopoietin. TAFI – thrombin activatable fibrinolysis inhibitor, tPA – tissue plasminogen activator, PAI-I – plasminogen activator inhibitor-I.

Antiplatelet drugs in liver fibrosis

Animal studies

Several studies conducted in the rodent model have suggested the possible mechanisms of antiplatelet drugs on liver fibrosis progress. In the mice model of chronic hepatitis B, dual antiplatelet therapy (DAPT): acetylsalicylic acid (ASA) + clopidogrel treatment combination – significantly reduced liver accumulation of viral-specific CD8 + T cells. The CD8 + T cells along with nonspecific inflammatory cells are responsible for immune-mediated hepatic injury leading to cirrhosis and HCC development. The treatment with antiplatelet agents in chronic hepatitis in mice was associated with a decreased collagen deposition in the liver parenchyma, further confirmed by transient elastography liver stiffness (TE-LS) values results. Moreover, DAPT correlated with delayed HCC progression and improved overall survival. This antitumor effect was observed only in viral-related HCC, not in the non-immunological hepatotoxicity mice model [45]. In the rat model of liver fibrosis induced by thioacetamide administration of ASA was correlated with dose-dependent fibrosis reduction. Furthermore, the anti-fibrotic effect was also observed in rats treated with enoxaparin (anticoagulative drug acting of factor Xa), which indicated coagulation as a potential mechanism responsible for liver fibrosis [46]. The relationship between induced liver fibrosis in rats, HSCs activation and coagulation system were studied by Mahmoud et al. The authors observed that platelet reactivity inhibition caused by clopidogrel administration resulted in a decrease of fibrosis markers and significant normalization in histopathological and biochemical changes. In the treated group, expression of α-smooth muscle actin marker (α-SMA), a biomarker of HSCs activation showed reduction compared with the carbon tetrachloride intoxicated control group [47]. The same results were reported in a group treated with dabigatran, an oral anticoagulation drug acting directly on clotting factor IIa (thrombin).

The animal studies indicated two pathways in which antiplatelet drugs may inhibit fibrosis. It was suggested that the key to preventing fibrosis is the modulation of immune response and inhibition of blood coagulation pathways. Thrombosis in liver microcirculation leads to local hypoxia, apoptosis and results in tissue damage and activation of the immune response. Both mechanisms are potential targets for antiplatelet drugs. The recent findings showed that ASA may target a P4HA2 enzyme, which is important in collagen formation and ECM accumulation. In the mouse xenograft model, ASA treatment resulted in the inhibition of this enzyme and a decrease of HCC tumor growth [45,48].

Human studies

ASA and liver fibrosis

Antiplatelet therapy is not a standard treatment in chronic liver diseases. Patients with thrombocytopenia and clotting disorders are at higher risk of bleeding complications.

Nevertheless, several patients with liver disorders and comorbidities like cardiovascular diseases (CVD) or neurovascular diseases receive antiplatelet drugs when its benefits outweigh the risk. Results of published studies may indirectly provide data of antiplatelet drugs influence on liver fibrosis however those studies were not designed to assess such effect.

In the group of nonalcoholic fatty liver disease (NAFLD) patients treated with ASA, $P2Y_{12}$ inhibitors or DAPT, antiplatelet therapy was inversely correlated with liver fibrosis occurrence or progression. This relation was dose-dependent, pronounced inhibition of fibrosis was observed in patients treated with DAPT including clopidogrel, ticagrelor or prasugrel compared to monotherapy with ASA [14]. However, the study did not compare the effect of ASA with $P2Y_{12}$ inhibitors or the effectiveness of distinct $P2Y_{12}$ inhibitors. Simon et al. studied patients with liver fibrosis receiving ASA due to either CVD or pain treatment. Assessment of liver fibrosis included noninvasive indices (FIB-4, NAFLD fibrosis score, and APRI) as well as liver biopsy. Daily ASA administration was associated with duration-dependent reduced risk of liver fibrosis progression and this effect was more pronounced in regular ASA users in the ten years follow-up period. The positive effect of ASA was still noticeable despite the older age of the treated group, which was followed by accompanying diseases and higher overall drug consumption. However, non-ASA nonsteroidal anti-inflammatory drugs (NSAID) e.g. ibuprofen or naproxen did not reduce the risk of liver fibrosis [15]. These findings might be explained by differences in the actions of ASA and non-ASA NSAIDs on cyclooxygenase (COX) isoforms. NSAIDs inhibit mostly COX-2, while low dose ASA affects COX-1 in platelets. Importantly,, ASA is an irreversible COX inhibitor, which may additionally strengthen the antifibrotic effect, observed in the study.

A large cross-sectional analysis also found a negative correlation between the use of ASA and liver fibrosis in the National Health and Nutrition Examination Survey III (NHANES III) participants. In this study, the patients with suspected chronic liver disease were identified by analysis of their medical history. The degree of liver fibrosis was determined with four validated fibrosis indices. The association between ASA and liver fibrosis was significant in subjects with suspected chronic liver disease. Moreover, it was consistent between four different origins of fibrosis. The results did not differ between frequent (≥15 doses per month) and occasional (<15 per month) ASA users, that have been observed in other research [15]. Besides, there was no association between the progression of fibrosis and other NSAID - ibuprofen which is in line with previous findings. These observations were further confirmed by the study focused on a group of patients with recurrent hepatitis C after liver transplantation, treated with ASA for primary prevention of hepatic artery thrombosis. Daily administration correlated with slower progression of fibrosis in histological assessment irrespective of immunosuppressive regimen [16,49]. It was reported that in the group of patients with type 2 diabetes mellitus (T2DM) and NAFLD, the administration of ASA had no protective effect against advanced fibrosis development [50]. It may be due to the fact that T2DM is a condition with chronic inflammation, wherein platelet function is increased [51,52]. Many factors, e.g. chronic proinflammatory status, hyperglycemia and the increased production of pro-oxidant species may act as platelet activators [53]. Thus, in T2DM patients an impaired response for antiplatelet treatment, both ASA and $P2Y_{12}$ receptor antagonists, was observed more often than in subjects without this ailment [54,55]. Moreover, it was shown that standard once-daily ASA administration did not affect platelet reactivity in T2DM patients, whereas twice-daily might reduce it significantly [56]. The findings indicate that inhibition of fibrosis is based on antiplatelet effect, but a modified, specially tailored drug regimen may be necessary to assess the effect of ASA in patients with T2DM.

There are at least three explanations of the ASA effect on chronic liver diseases. First, ASA may prevent arterial thrombosis and subsequently reduce the risk of venous thromboembolism [57]. Thrombosis in intrahepatic microcirculation appears to be one of the most important pro-fibrotic factors which along with the sinusoidal cells damage leads to increased reactivity and adhesion of platelets. It is well-known that low dose (75–150 mg) ASA used chronically has potent antiplatelet activity with weak anti-inflammatory effect [58]. Second, ASA may inhibit the release of several mediators by activated platelets, such as

PDGF-β directly activating HSCs, and extracellular microvesicles which play a key role in cellular interactions [59,60]. This may explain the observed effectiveness of low dose ASA in detaining liver fibrosis progression. Cheng et al. revealed that ASA may block the pro-inflammatory, pro-coagulant, and adhesion properties of platelet-derived microvesicles via modulation of ERK-NO /O2- and p38-NF-κB-VCAM-1 signaling cascades [61]. Further studies focused on the molecular mechanism of platelet reactivity and its significance in cellular crosstalk may explain the role of the antiplatelet treatment in chronic liver diseases. Third, ASA induces anti-inflammatory, endogenous lipids known as 'aspirin-triggered lipoxins' (ATLs). ATLs are lipoxins 15-epimers, derived by the arachidonic acid metabolism (in this pathway acetylsalicylic acid is required for COX-2 acetylation). They are expressed by platelets, leukocytes and endothelial cells and promote silencing of inflammation [62]. In addition, ATLs synthetic analog decreased TGF-β and VEGF expression, inhibited collagen deposition and reversed fibrosis in the mouse model of bleomycin-induced pulmonary fibrosis [63,64]. Paradoxically, other NSAIDs may not have the same influence on arachidonic acid metabolism, which can explain why some authors did not observe their antifibrotic effect [65] (Figure 2, Table 1).

ASA and HCC risk

Around 9% of HCC cases develop on the background of liver cirrhosis therefore one of the therapeutic goals in liver cirrhosis is the prevention of HCC [73]. It was found that the risk of HCC in ASA treated patients was 49% lower than in non-users. Moreover, the risk of death due to chronic liver diseases was 50% lower in ASA treated group [69]. Analysis of two large prospective cohort studies comprising 133 371 subjects implied that long-term ASA treatment is correlated with a dose- and duration-dependent HCC risk reduction. Similar to the above-mentioned fibrosis studies, the treatment of NSAIDs other than ASA had no impact on HCC occurrence [71]. In line with those important findings, Hwang et al. in the observational study conducted in a cohort of 460 755 participants living in a region of high viral hepatitis prevalence, reassured the protective role of ASA in HCC development. On the contrary to the previous study, the positive effect was enhanced by other NSAIDs administration, especially in viral hepatitis [67]. Moreover, in patients with viral hepatitis-related cirrhosis ten years' follow-up showed that daily treatment of low dose ASA reduced the incidence of HCC and improved the overall survival. As observed in previous studies, the effect was duration-dependent and the greatest benefits were achieved after at least two years of daily use of ASA [66]. It was also demonstrated that ASA used as chemoprophylaxis after surgical tumor resection, significantly reduced the risk of HCC recurrence in patients with viral hepatitis B [72].

In the group of 10 615 patients with chronic hepatitis B living in Taiwan, patients who received ASA therapy were randomly matched 1:4 with subjects who had never received ASA. The cumulative incidence of HCC in 5 years was significantly lower in the treated group, receiving ASA daily for at least 90 days, compared to the untreated group [68]. Although most studies have been conducted in the viral hepatitis cohorts, recent reports have shown that ASA treatment was also associated with a reduction in the risk of developing HCC in patients with alcoholic cirrhosis [70]. Thus, ASA treatment may be effective in the reduction of the HCC risk regardless of etiology (Figure 2, Table I).

$P2Y_{12}$ receptor antagonists

The $P2Y_{12}$ antagonists seem to be possible therapeutic agents in liver fibrosis not only due to its antiplatelet properties. Previous studies showed that ticagrelor may decrease levels of pro-inflammatory cytokines – TNF and IL-6 [74,75]. Both antiplatelet and antiinflammatory mechanisms of ticagrelor may be beneficial for inhibition of liver fibrosis. However, the data focused on $P2Y_{12}$ inhibitors in liver disorders are limited. Up to date, only one study assessed $P2Y_{12}$ receptor antagonists in a cohort of patients at risk of cardiovascular events with NAFLD (n = 134), however the cohort receiving only $P2Y_{12}$ inhibitors without ASA was relatively small (n = 26). The most important finding from the study was a negative correlation between the use of antiplatelet agents and stage of fibrosis. Besides, there was a significant correlation between platelet counts and the stage of liver fibrosis defined by FibroScan transient elastography test. Moreover, the ratio of PDGF-β to platelets was significantly higher in patients with liver fibrosis compared to patients without liver fibrosis. However, PDGF-β serum concentration did not differ between patients who were treated or not with antiplatelet agents. Importantly, the impact of other drugs cannot be excluded, as in this study, patients on antiplatelet agents were more frequently on statin treatment, which may bias the effect of antiplatelet agents on liver fibrosis. Hence, randomized trials of antiplatelet agents in the prevention of liver fibrosis progression are awaited [14] (Figure 2, Table I).

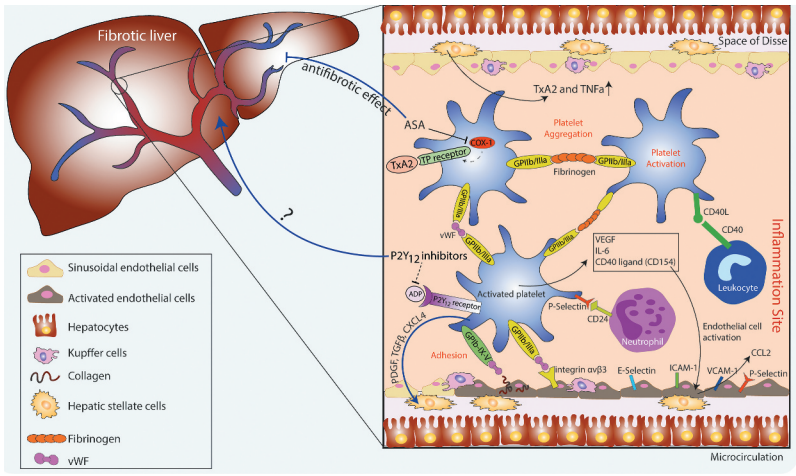

Figure 2. **Antiplatelet treatment in liver fibrosis. Fibrotic liver and microcirculation**; Activated platelets stimulate the production of mediators including, PDGF, TGF-β and CXCL4 which stimulate the activation of hepatic stellate cells (HSCs). Activated HSCs increases collagen production, extracellular matrix deposition and fibrosis. Antiplatelet treatment inhibits the platelet activation thus may ameliorate this process. Kupffer cells (KCs) activated by liver injury are the main source of pro-inflammatory cytokines, for example TNF-α. It activates HSC and endothelial cells, which leads to inflammation, liver damage and aggravate fibrogenesis. KCs release thromboxane A2 (TXA2) - vasoconstrictive eicosanoid stimulating leukocyte adhesion, platelet aggregation as well as the synthesis of TNF-α. ASA treatment irreversibly inhibits platelet cyclooxygenase 1 (COX-1), and TXA2. Therefore, it can show an antifibrotic effect in liver fibrosis. However, the effect of $P2Y_{12}$ inhibitors on liver fibrosis is unknown.

Table 1. Studies on antiplatelet treatment on the risk and hepatocellular carcinoma (including observational studies and cross-sectional research) in patients treated with ASA, $P2Y_{12}$ antagonists or both due to diseases other than hepatic impairment.

Author	Number of patients	ASA group	Antagonists of $P2Y_{12}$ receptor group	Type of liver disease	Effect on fibrosis	Risk of HCC
Du (2019) [66]	264	+	n/d	Viral hepatitis	n/d	↓
Hwang (2018) [67]	460 755	+	n/d	All etiologies	n/d	↓
Jiang (2016) [49]	1907	+	n/d	Viral hepatitis, alcohol abuse, NASH	↓	n/d
Lee (2019) [68]	10 615	+	n/d	Viral hepatitis	n/d	↓
Poujol – Robert (2014) [16]	188	+	n/d	Viral hepatitis	↓	n/d
Sahasrabuddhe (2012) [69]	300 504	+	n/d	All etiologies	n/d	↓
Schwarzkopf (2018) [14]	505	+	+	NAFLD	↓	n/d
Shin (2020) [70]	949	+	n/d	Alcoholic cirrhosis	n/d	↓
Simon (2018) [71]	133 371	+	n/d	All etiologies	n/d	↓
Simon (2019) [15]	361	+	n/d	NAFLD	↓	n/d
Singh (2018) [50]	592	+	n/d	NAFLD	↔	n/d
Young (2019) [72]	430	+	n/d	Viral hepatitis	n/d	↓

Abbreviations: ASA - acetylsalicylic acid, HCC - hepatocellular carcinoma, NAFLD - nonalcoholic fatty liver disease, NASH - nonalcoholic steatohepatitis, n/d – no data, ↓ - decreased, ↔ - no effect

Antiplatelet treatment and the risk of portal vein thrombosis

The Virchow's triad - hypercoagulability, reduced portal flow, and endothelial injury is present in liver cirrhosis, increasing the risk of thrombotic complications. Portal vein thrombosis (PVT) is a potentially lethal complication which may accelerate disease progression and worsened patients prognosis. Antiplatelet therapy in PVT prevention was studied in cirrhotic patients after splenectomy. Splenectomy is associated with increased platelet count and higher risk of PVT. Post-splenectomy ASA (300 mg/d) treatment administered for three months in patients with postoperative platelet count (PLT) exceeded 300×10^9/L did not decrease the risk of PVT [76]. On the contrary, Zhou et al. proved that early initiation of antiplatelet therapy can be beneficial in patients after splenectomy with gastroesophageal devascularization, which is associated with the highest risk of thrombotic complications. Patients treated with lower dose of ASA (100 mg/d; administered when PLT exceeded 200×10^9/L) and dipyridamole had a significantly reduced PVT risk when compared with group received antiplatelet therapy when PLT exceeded 300×10^9/L. Moreover, in long-term observation no major bleeding were observed [77]. Thus, antiplatelet therapy may be beneficial in PVT but appropriate dosage, duration of the therapy and target patient groups have to be defined. Cirrhotic patients may be at increased risk of deep vein thrombosis (DVT), but the impact of antiplatelet therapy on DVT risk has not been assessed in this group [78].

Safety of antiplatelet drugs in chronic liver diseases

Any potential benefits of antiplatelet therapy must be weighed against bleeding risk especially in patients with chronic liver diseases. Antiplatelet treatment is a known risk factor of gastrointestinal bleeding (GIB) in the overall population [79,80]. In the group of low-dose ASA users, the incidence of lower GIB within 1 year was significantly higher than in the non-ASA control group (0.20% vs 0.06%) [80]. Liver fibrosis is accompanied by coagulation disorders. Besides, the risk of bleeding in patients with chronic liver disease may be increased by portal hypertension [81]. In cirrhotic patients treated with DAPT, the incidence of GIB was 22.1%, compared with 5% in the non-cirrhotic controls. Nonetheless, survival was comparable in treated and non-treated groups. None of the patients required surgery or angiography to maintain hemostasis, and there were no deaths due to gastrointestinal bleeding [82]. In another study, DAPT showed effectiveness in secondary prophylaxis of myocardial infarction in patients with cirrhosis but in line with previous reports was significantly correlated with increased risk of GIB. A similar risk of bleeding was observed during single antiplatelet therapy [83]. In cirrhotic patients treated with ASA as secondary prophylaxis of ischemic stroke, the risk of readmission due to the upper GIB was not increased [84]. Similarly, the frequency of peptic ulcer bleeding in patients with chronic hepatitis B was not higher in the ASA-treated group but the risk may be underestimated due to the poor drug tolerance in the studied group [68].

Antiplatelet drugs during liver biopsy and surgery

Liver biopsy is still considered as a diagnostic gold standard in staging of liver fibrosis. However liver biopsy is an invasive procedure with numerous disadvantages [85,86]. One of the main limitations of the procedure is increased bleeding risk in patients on antiplatelet therapy. Although studies have shown the increased frequency of bleeding in patients taking drugs interfering with platelet function [87], there are no clearly clarified guidelines for patients requiring liver biopsy during antiplatelet treatment. Normal platelet lifespan is 7–10 days, and in clinical practise, it is common to pause the antiplatelet agents approximately 7 days before elective procedures to decrease the bleeding risk. Latest British guidelines recommend to interrupt clopidogrel treatment 7 days and ASA 3–7 days before liver biopsy. On the other hand, it was also recommended that patients receiving DAPT after coronary stents implantation should be considered to stop taking $P2Y_{12}$ inhibitors, but ASA treatment should always continue [88].

It is important to note that the assessment of the hemostatic function in cirrhosis is challenging. The traditional coagulation tests like prothrombin time (PT), the international normalized ratio (INR), and activated partial thromboplastin time (aPTT) are widely used for estimating the risk of

bleeding and determination of the best treatment. These tests validate only the procoagulant capacity [89]. In the injured liver production not only pro- but also anticoagulant factors are altered, and assessment of deviations of PT, aPTT, and INR may not provide a reliable prediction for bleeding risk in cirrhotic patients [90–92]. Therefore, as it was reviewed deeply by Sharma et al., to overcome the drawbacks of liver biopsy, several noninvasive techniques have been investigated for the assessment of fibrosis [85,86]. Radiologic techniques and serum-based markers are considered noninvasive methods. Radiologic techniques include ultrasound, magnetic resonance imaging and elastography (transient elastography and magnetic resonance elastography). Serum-based biomarkers of cirrhosis include ARPI, Fibrotest, FIB4 panel, NAFLD fibrosis score, Fibroindex, as well as promising markers, such as hyaluronic acid, N-terminal of serum procollagen III peptide (PIINP), TIMP-1, YKL-40 (chondrex) and ELF score [93–96].

Limited studies aimed to analyze the efficacy and safety of antiplatelet drugs after liver transplantation. Previous study documented that long-term ASA treatment is associated with decreased incidence of hepatic artery thrombosis, whereas is not associated with bleeding complications in patients after liver transplantation [97,98]. Another study showed that ASA administration immediately after liver transplantation is safe and effective in decreasing early hepatic artery thrombosis, with no excessive bleeding risk [99].

The cirrhotic patients are at risk of thrombocytopenia and bleeding disorders, during invasive procedures. Usage of TPO agonists before intervention increases platelet count and may avoid platelet transfusion, however, the specific influence on bleeding risk is still unexplained [100]. The ELEVATE study assessing the efficacy of first-generation TPO agonist eltrombopag was early stopped due to increased risk of PVT in the treated group. Statistical analysis showed an association between PVT events and a platelet count of over 200 × 10^9/L [101]. The safety profile of second-generation TPO agonists dedicated to perioperative management of CLD patients was similar to placebo [102,103]. Avatrombopag increased platelet count but did not activate platelets [104]. Platelet activation is essential for thrombotic complications, thus, it can be suspected that the thrombotic effect of periprocedural second-generation TPO treatment is negligible. The duration of therapy is short (5–7 days) therefore, significant effect of the TPO on fibrosis is not expected [103,105].

Conclusions

The pathophysiology of liver fibrosis is complex. Platelets play a pivotal role in the mechanisms involved in disease progression. Their inhibition seems to be a promising therapeutic target. The results of previous observational studies show that ASA treatment was correlated with a decrease in liver fibrosis progression as well as HCC development. However, chronic liver diseases are also associated with the increased risk of bleeding which can be aggravated during antiplatelet therapy. Therefore the practical aspects of antiplatelet therapy in liver fibrosis and indications for its implementation should be elucidated. Further research is necessary to; i) define the factors allowing to stratify the risk of bleeding in the group of chronic liver disease patients, ii) find patients who can benefit most from the treatment, iii) assess the real advantages of antiplatelet treatment in chronic liver diseases. Moreover, the safety and efficacy of antiplatelet treatment should be assessed in randomized clinical trials which may be also helpful to exclude other factors which may be the bias of observational studies.

Abbreviations

ADP – adenosine diphosphate;
APRI – AST to platelet ratio;
aPTT – activated partial thromboplastin time;
ASA – acetylsalicylic acid;
ATLs – aspirin-triggered lipoxins;
ATP – adenosine triphosphate;
cAMP – cyclic adenosine monophosphate;
CCL2 – chemokine ligand 2;
CD40 – cluster of differentiation 40;
CD154 – CD40 ligand;
CLD – chronic liver disease;
COX – cyclooxygenase
COX-1 – cyclooxygenase 1;
COX-2 – cyclooxygenase 2;
CVD – cardiovascular diseases;
DAPT – dual antiplatelet therapy;
DVT – deep vein thrombosis;
ECM – extracellular matrix;
ELF – Enhanced Liver Fibrosis;
ENT1 – equilibrative nucleoside transporter 1;
HCC – hepatocellular carcinoma;
HGF – hepatic growth factor;
HSCs – hepatic stellate cells;
FIB-4 – fibrosis 4 index for liver fibrosis;
GIB – gastrointestinal bleeding;
ICAM-1 – intercellular adhesion molecule 1;
I-COMET – Intercontinental Cardiovascular and Cardiometabolic Research Team;
IL-6 – interleukin 6;
IL-33 – interleukin 33;
INR – international normalized ratio;
KCs – Kupffer cells;
LSECs – liver sinusoidal endothelial cells;
MCP1 – monocyte chemoattractant protein-1;
NAFLD – nonalcoholic fatty liver disease;
NHANES III – National Health and Nutrition Examination Survey III;
NSAID – nonsteroidal anti-inflammatory drug;
$P2Y_{12}$ – purinergic receptor;
P4HA2 – prolyl 4-hydroxylase subunit alpha-1;
PIINP – N-terminal of serum procollagen III peptide;
PAF – platelet activating factor;
PAI-I – plasminogen activator inhibitor-I;
PDGF – platelet-derived growth factor;
PLT – platelet count
PT – prothrombin time;
PVT – Portal vein thrombosis;
α-SMA – α-smooth muscle actin marker;
STAT3 – signal transducer and activator of transcription 3;
T2DM – type 2 diabetes mellitus;
TAFI – thrombin activatable fibrinolysis inhibitor;
TE-LS – transient elastography liver stiffness test;
TF – tissue factor;
TFPI – tissue factor pathway inhibitor;
TGF-β – transforming growth factor beta;
TIMP-1 – tissue inhibitor of metalloproteinases;
TNF – tumor necrosis factor;
tPA – tissue plasminogen activator;

TPO – thrombopoietin;
TXA2 – thromboxane A2;
T2DM – type 2 diabetes mellitus;
VCAM-1 – vascular cell adhesion molecule 1;
VEGF – vascular-derived growth factor;
vWF – von Willebrand factor;
YKL-40 – chitinase-3-like protein 1.

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Author contributions

PC, CE contributed to the data collection and elaboration, writing and approval of the manuscript; and is the guarantor of the article. AN, MW, MP, AP, AC, DMG contributed discussion and writing and approval of the manuscript. AP, AC contributed valuable revision of the manuscript. PC and CE contributed valuable contributions to graphical designs. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Conflicts of interest

The authors state there are no conflicts of interest.

ORCID

Pamela Czajka http://orcid.org/0000-0002-0193-0192
Adam Przybyłkowski http://orcid.org/0000-0002-5851-2856
Anna Nowak http://orcid.org/0000-0001-9158-1055
Marek Postula http://orcid.org/0000-0002-7826-4458
Marta Wolska http://orcid.org/0000-0002-3190-8113
Dagmara Mirowska-Guzel http://orcid.org/0000-0001-6294-3256
Anna Czlonkowska http://orcid.org/0000-0002-1956-1866
Ceren Eyileten http://orcid.org/0000-0002-3324-9625

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