Review

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A focus on the role of platelets in liver regeneration: Do platelet-endothelial cell interactions initiate the regenerative process?

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Summary

Platelets are involved in the early phases of liver regeneration. Moreover, platelet transfusion and thrombocytosis were recently shown to enhance hepatocyte proliferation. However, the precise mechanisms remain elusive. This review discusses the latest updates regarding the mechanisms by which platelets stimulate liver regeneration, focusing on their interactions with liver sinusoidal endothelial cells and on their fate within the liver.

Following liver injury, platelets are recruited to and trapped within the liver, where they adhere to the endothelium. Subsequent platelet activation results in the release of platelet granules, which stimulate hepatocyte proliferation through activation of the Akt and ERK1/2 signalling pathways. Platelets activate liver sinusoidal endothelial cells, leading to the secretion of growth factors, such as interleukin-6. Finally, liver sinusoidal cells and hepatocytes can also internalize platelets, but the effects of this alternate process on liver regeneration remain to be explored. A better understanding of the mechanisms by which platelets stimulate liver regeneration could lead to improvement in post-operative organ function and allow hepatectomies of a greater extent to be performed.

Keywords: Hepatocyte; Proliferation; Liver sinusoidal endothelial cell; Endothelium; β2-integrin; Macrophage-1 antigen; Lipopolysaccharide; Akt; ERK1/2; Signaling pathway; Interleukin-6; Internalization.

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Abbreviations: PH, partial hepatectomy; LSEC, liver sinusoidal endothelial cell; TGF-β, transforming growth factor-β; IL-6, interleukin-6; EGF, epidermal growth factor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; LPS, lipopolysaccharide; VEGF, vascular endothelial growth factor; MAC-1, macrophage-1 antigen.

Introduction

First studied for their haemostatic properties, platelets were later shown to exhibit significant inflammatory and immunomodulatory properties. Their α-granules contain a large range of haemostatic factors, cellular mitogens and adhesion molecules. Their dense granules harbour nucleotides, calcium and serotonin, which are involved in platelet activation and recruitment, and their lysosomes contain proteases and hydrolases [1]. By releasing these molecules or presenting them on their surface, platelets interact with other cell types and contribute to wound healing [2], immune modulation [3] and cell proliferation [4].

Key points

- Following liver injury, platelets are recruited to the liver and adhere to liver sinusoidal endothelial cells. Some of them migrate into the space of Disse and make direct contact with hepatocytes.
- Upon contact with liver sinusoidal endothelial cells or hepatocytes, platelets release molecules that directly or indirectly stimulate hepatocyte proliferation.
- Platelets can be internalized by liver sinusoidal endothelial cells or hepatocytes.

In 1996, platelets were first reported to be beneficial to hepatocyte proliferation after hepatectomy [5]. When induced by thrombopoitein, thrombocytosis accelerated liver regeneration after partial hepatectomy (PH) [6–9] and improved survival following subtotal resection [8] in rodents. A similar effect was observed after splenectomy [5]. In addition, platelet-rich plasma transfusion, considered to be a promising novel therapy for patients undergoing liver resection, promoted liver regeneration

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in rats after 70% PH [10]. Conversely, thrombocytopenia, induced by either the administration of anti-platelet antibodies [7,11] or the thrombocytolytic drug busulfan [11], depressed liver regeneration.

However, the mechanisms by which platelets exert their proliferative effect remain elusive. This review discusses the potential mechanisms by which platelets stimulate liver regeneration, focusing on their interactions with liver sinusoidal endothelial cells (LSEC) and on their fate within the liver.

Platelets promote liver regeneration by activating the Akt and ERK1/2 signalling pathways in hepatocytes

The signalling pathways leading to hepatocyte proliferation are numerous and controlled by a variety of growth factors, hormones and cytokines that direct every step of the regeneration process [12,13].

Transforming growth factor-β (TGF-β), the main terminator of hepatocyte proliferation [12], induces apoptosis in hepatocytes *in vitro* [14,15] and *in vivo* [16]. By contrast, no cell death occurs after PH, suggesting that the apoptotic pathways are suppressed during liver regeneration. This was confirmed by Hong *et al.*, who demonstrated that the anti-apoptotic signalling pathway Akt was strongly activated in hepatocytes after PH, as well as after *in vitro* and *in vivo* exposures to insulin, phenylephrine, interleukin-6 (IL-6), epidermal growth factor (EGF), and hepatocyte growth factor (HGF), which are all known mitogens or co-mitogens for hepatocytes. Moreover, insulin and phenylephrine rescued hepatocytes from TGF-β-induced apoptosis *in vitro*. These effects were antagonized by the phosphatidylinositol 3-kinase inhibitor LY294002 [17], suggesting that the Akt pathway protects hepatocytes from apoptosis during the regeneration process.

Matsuo *et al.* later reported that the co-culture of platelets and hepatocytes activated both the Akt pathway and the extracellular signal-regulated kinases ERK1/2 within 1 and 10 min, respectively, but not STAT3, which is primarily controlled by IL-6. Platelets exerted a direct proliferative effect on cultured hepatocytes, as demonstrated by increased [3H]-methyl-thymidine and bromodeoxyuridine uptakes [18]. *In vivo* studies confirmed that rats transfused with platelets exhibited earlier Akt pathway activation, concomitant with that of the ERK1/2 pathway, and greater liver/body weight ratios. Ki67 immunohistochemistry also demonstrated increased hepatocyte proliferation [10]. Furthermore, Murata *et al.* showed that following Kupffer cell depletion, liver regeneration was delayed because of lower tumour necrosis factor-α expression but was restored by thrombocytosis with levels of HGF, insulin-like growth factor-1 (IGF-1) and Akt phosphorylation higher than those of controls [19]. Other authors confirmed that thrombocytosis induced a strong activation of the Akt pathway in hepatocytes following PH [7,8].

Platelet are recruited to the sinusoidal and Disse's spaces following systemic inflammatory stimulus and partial hepatectomy

Endo *et al.* showed that endotoxemia, generated by administering gut-bacterial lipopolysaccharide (LPS) to mice, induced a dose-dependent increase in the hepatic content of serotonin, associated with both a dose-dependent decrease in circulating platelets and a reduction in the blood concentration of serotonin [20]. As most of the peripheral serotonin is contained in platelets [21], this finding suggests that platelets accumulate or release their content in the liver. The administration of other cytokines involved in the early phase of liver regeneration, such as interleukin-1 and tumour necrosis factor-α, had the same effect on platelets. Electron microscopy revealed that, in animals treated with LPS, numerous platelets were trapped in the sinusoidal and Disse's spaces with no evidence of degranulation, suggesting that LPS and cytokine-induced mobilisations of serotonin in the liver were associated with the hepatic translocation of inactivated platelets [20]. Nakamura *et al.* later confirmed via electron microscopy that 10–20% of platelets accumulated in the liver 4.5 h after LPS injection, some of which were found in the space of Disse [22].

Similar recruitment of platelets to the liver occurred after liver surgery without ischemia–reperfusion injury. Studies with an *ex vivo* liver perfusion porcine model demonstrated a profound drop in platelet count after liver tissue manipulation and in the immediate post-operative phase of a PH, associated with an increased platelet activation, as illustrated by elevated plasma levels of platelet factor-4 and β-thromboglobulin (factors that are releasates of platelet α-granules and constitute markers of platelet activation) and electron microscopy [23]. The authors hypothesized that the early acute thrombocytopenia occurring after PH might be due to platelet local activation and trapping within the liver. Other authors recently confirmed by immunohistochemistry [19] and intravital videomicroscopy [10] that following PH, platelets accumulated in the sinusoids. Moreover, through electron microscopy, platelets were observed translocating into the space of Disse and making direct contact with hepatocytes as early as 5 min after surgery [19]. LSEC fenestrae were described to have increased diameters within the same time

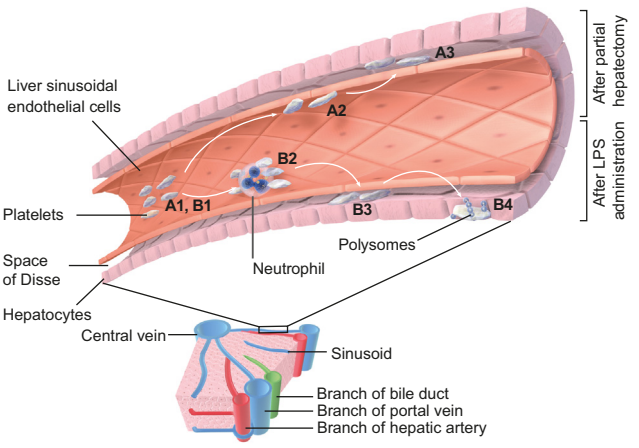

Fig. 1. The fate of platelets within the liver sinusoids following partial hepatectomy or systemic inflammatory stimulus by lipopolysaccharide. Following partial hepatectomy, platelets are recruited to the sinusoids (A1) [10,19]. They roll over and adhere to liver sinusoidal endothelial cells (A2) [10]. Some platelets translocate into the space of Disse and make direct contact with hepatocytes (A3) [19]. Following lipopolysaccharide administration, platelets are recruited to the liver sinusoids and remain trapped within the liver (B1) [20,22]. Neutrophils adherent to liver sinusoidal endothelial cells serve as a support onto which platelets bind and aggregate, forming microthrombi that occlude the sinusoids (B2) [44,45]. Some platelets translocate into the space of Disse (B3) [20,22], where they are internalized by hepatocytes and surrounded by polysomes (B4) [22]. LPS: lipopolysaccharide.