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Mini Review

VEGF/VEGFR signaling in the liver repair from acetaminophen hepatotoxicity

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Acetaminophen (APAP) hepatotoxicity because of overdose is the most frequent cause of acute liver failure. The mechanisms of APAP hepatotoxicity are dominated by intracellular events including the formation of a reactive metabolite, hepatic glutathione depletion and protein binding. In response to overdose of APAP treatment, the liver elicits a healing process characterized by proliferation of hepatocytes, removal of necrotic tissue, and restoration of the hepatic microvasculature. However, the mechanisms of repair of the tissue damage during APAP hepatotoxicity are poorly understood. Vascular endothelial growth factor (VEGF) and its receptors, VEGFR1 and VEGFR2, promote the repair and regeneration of the liver after acute insult including liver resection and toxicants. This mini review focuses on the role of VEGF/VEGFRs signaling in liver injury and hepatic tissue repair during APAP hepatotoxicity.

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Introduction

Acetaminophen (N-acetyl-para-aminophenol) (APAP) is a commonly used, over-the-counter analgesic and antipyretic with few side effects when taken at therapeutic doses. However, APAP toxicity from an overdose can result in severe hepatic damage in both humans and animals[1)]. Metabolic activation of APAP and protein adduct formation, mitochondrial dysfunction, oxidant stress, peroxynitrite formation and nuclear DNA fragmentation are critical intracellular events in hepatocytes[2, 3)]. Although the research in understanding the mechanisms of APAP-induced liver injury has been focused on intracellular events in hepatocytes, there also is an increasing awareness that infiltrating inflammatory cells are involved in the pathogenesis[4-6)]. Furthermore, hepatic microcirculatory dysfunction contributes to the liver injury elicited by APAP[7-10)].

In addition to the injury mechanisms, initiation of regeneration is critical for the repair of the damaged liver tissue and the resolution of the inflammation[11)]. In response to toxin-induced acute liver injury, the liver elicits a healing process characterized by proliferation of hepatocytes, removal of necrotic tissue and matrix remodeling leading to restoration of a normal hepatic structure. However, the underlying mechanisms of liver repair process appear to be complex and unclear[5, 6, 12)].

Vascular endothelial growth factor (VEGF)-A is a major

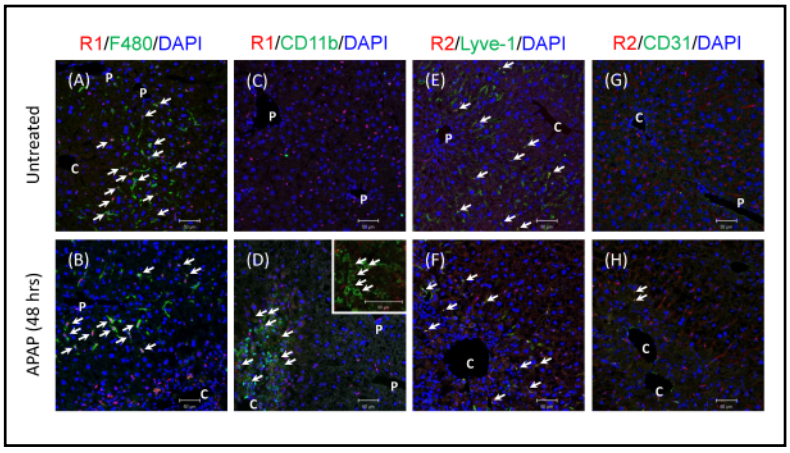

Fig.1 Liver expression of VEGFR1 and VRGFR2 after APAP treatment

Double staining of liver sections from WT with antibodies against VEGFR1 (red) and F4/80 (green), VEGFR1 (red) and CD11b (green), VEGFR2 (red) and Lyve-1 (green) or VEGFR2 (red) and CD31 (green). A, C, E and G, double labeling in untreated livers; B, D, F and H, double labeling in livers treated with APAP for 48 h. Arrows indicate double-labeled cells. DAPI staining is shown to identify cell nuclei (blue). C, central vein; P, portal vein. Scale bars indicate 50 μm.

regulator of development, and of physiological and pathological angiogenesis[13, 14]. VEGF acts primarily through two tyrosine kinase receptors, VEGF receptor-1 (VEGFR1, flt-1) and VEGF receptor-2 (VEGFR2, flk-1, kdr). VEGF-A binds to VEGFR1 with a 10-fold higher affinity than to VEGFR2, though the tyrosine kinase activity of VEGFR1 is relatively weak. Although both receptors are expressed in endothelial cells, VEGFR1 is also expressed in monocytes/macrophages[14, 15]. VEGF-induced angiogenesis is mainly mediated by VEGFR2 activation[13, 16]. In contrast, the biological role of VEGFR1 is highly complex. Although genetic data indicate that signaling downstream of this receptor is not required for developmental angiogenesis[17], a role for VEGFR1 during tumor-angiogenesis has been recently suggested[18, 19]. VEGFR1 signaling also implicates the recruitment of macrophages in the inflammatory sites[20].

In this mini review, we will present several studies including ours on the critical roles of VEGFR1 and VEGFR2 receptors in liver injury and repair of the liver tissue after APAP toxicity.

VEGF/VEGFR expression in the liver after APAP administration

The overdoses of APAP administration to mice causes a significant liver injury as evidenced by serum ALT activities, peaking at 24 h after APAP (injury phase), and returned to the normal levels within 48 h and thereafter APAP (repair phase). During APAP hepatotoxicity, the expressions of VEGF and its receptors, VEGFR1 and VEGFR2, are enhanced[21-23]. Although the time periods when VEGF expression is up-regulated during the course of APAP hepatotoxicity differ among these reports, the significant increases in VEGF protein levels are found in the late phase of injury, indicating a critical role of VEGF in the recovery from APAP hepatotoxicity. The enhanced expression of VEGF is demonstrated in hepatocytes during APAP hepatotoxicity[21]. The expression of VEGFR1 is localized in the sinusoids of untreated liver (Fig.1A and 1C). Double immunofluorescein analysis for identification of the sinusoidal cells expressing VEGFR1 reveals that these cells are positive for F4/80, a marker of resident macrophages (Kupffer cells) (Fig.1A and 1B). During APAP hepatotoxicity, a significant increase in hepatic VEGFR1 protein expression is peaked at 48 h after APAP[21]. At the same time point, VEGFR1-positive cells are accumulated in the injured centrilobular regions. These VEGFR1-positive cells in the injured area are negative for F4/80 (Fig.1B), but are positive for CD11b (Fig.1D), an indication for recruited macrophages[24]. The expression of VEGFR1 is not co-localized with CD31, a marker of endothelial cells[23]. On the other hand, in control livers, VEGFR2 is expressed along the sinusoids (Fig.1E to 1H). These VEGFR2-cells are positive for Lymphatic vessel endothelial hyaluronan receptor 1 (Lyve-1) (Fig.1E and 1F), an indicator for liver sinusoidal endothelial cells (LSECs) (25), but not for CD31 (Fig.1G and 1H). The administration of APAP causes an increase in hepatic protein levels of VEGFR2 expression from 8 through 48 h after[21]. Double immunofluorescein analysis reveals that VEGFR2-expressed cells are positive for Lyve-1 as well as CD31 48 h after APAP treatment (Fig.2). Collectively, enhanced VEGFR1 is expressed on the recruited macrophages, and VEGFR2 is expressed on the LSECs during the repair phase of APAP hepatotoxicity.

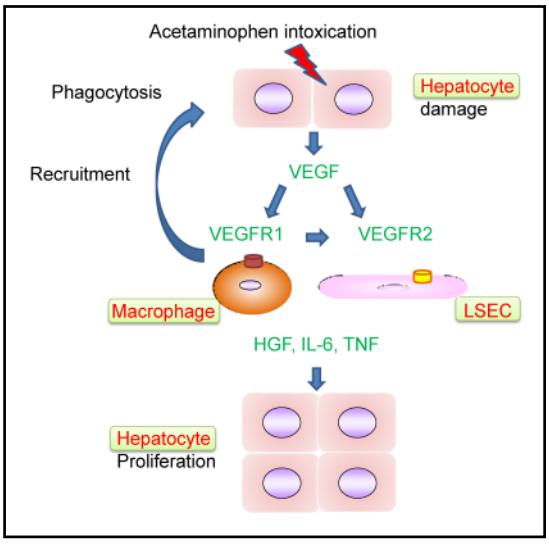

Fig.2 Schematic representation of the VEGF/VEGFR-mediated pathway for the enhancement of liver repair during acetaminophen hepatotoxicity

VEGF released from hepatocytes binds to recruited macrophage VEGFR1 to the damaged tissue to facilitate removal of dead cells and proliferation of hepatocytes through TNF and HGF production. VEGFR2 signaling in LSECs is involved in the restoration of the functional integrity of LSECs, eventually improving the sinusoidal perfusion.

Roles of VEGFRs during injury phase of APAP hepatotoxicity

Prior studies suggest that both VEGFR1 and VEGFR2 signaling appear not to be involved in liver injury during the course of APAP hepatotoxicity. For example, VEGFR2 signaling may not be responsible for acute liver injury, because the pharmacological interventions with VEGFR2 kinase inhibitors fail to protect against APAP hepatotoxicity[21, 23]. Additionally, there is no significant difference in the magnitude of liver injury between WT mice and VEGFR1 tyrosine kinase (TK)-deficient mice[23], indicating that VEGFR1 signaling also is not involved in APAP hepatotoxicity. Taken together, VEGF/VEGFR signaling pathway plays a minor role in APAP-induced liver injury. Nevertheless, our analyzes suggest that VEGF-VEGFR signaling plays a substantial role.

Roles of VEGFRs in macrophage recruitment during repair phase of APAP hepatotoxicity

We showed that VEGFR1-TK-deficient mice exhibit suppression of recruited VEGFR1 macrophages expressing CD11b[23]. Thus, VEGFR1 signaling plays a role in the recruitment of macrophages expressing VEGFR1/CD11b in the injured livers. Recent reports have revealed that macrophages accumulated in response to an APAP challenge represent a bone marrow-derived, circulating monocyte/macrophage population, distinct from resident Kupffer cells[24]. The newly recruited macrophages appear to be involved in cell debris removal during the later phase of APAP hepatotoxicity as a prerequisite for regeneration and replacement of necrotic cells[24, 26]. These data support the hypothesis that VEGFR1 signaling pathway contributes to the recruitment of macrophages expressing VEGFR1/CD11b, which play a key role in liver tissue repair process after APAP hepatotoxicity.

Increasing experimental evidence indicates that the infiltrating macrophages (M2), which are distinct from activated resident Kupffer cells (M1), are critical for the removal of necrotic cells and for tissue repair after APAP hepatotoxicity[5, 24]. In addition, the recruitment of M2 macrophages into the injured areas occurs through monocyte chemoattractant protein (MCP-1)/C-C chemokine receptor 2 (CCR2) signaling during APAP hepatotoxicity[24, 27]. Thus, it would be interesting to know whether characterization of VEGFR1/CD11b macrophages shares a phenotype with M2 macrophages and whether MCP-1 and its receptor, CCR2 signaling pathway is involved in the recruitment of macrophages expressing VEGFR1/CD11b.

Furthermore, neutrophils are recruited into the area of necrosis where they may participate in the healing and phagocytosis of cellular debris[12]. In contrast, M2 macrophages can induce apoptosis of neutrophils, which contributes to the resolution of the inflammatory response after APAP induced liver injury[24]. The role of neutrophils in tissue regeneration and its involvement of VEGFR1 signaling have not been specifically investigated.

Roles of VEGFRs in LSEC restoration during repair phase of APAP hepatotoxicity

VEGFR1-TK signaling is preventive from LSEC injury, because the hepatic hemorrhage in VEGFR1-TK-deficient mice is sustained during the late phase of APAP hepatotoxicity. The enhanced gap formation in LSECs and compromised endocytosis of LSECs mediated by the scavenger receptors also are shown in VEGFR1-TK-deficient mice. The formation of gaps in LSECs is caused by MMP-9 acti-

vation[7, 10, 23]. These gaps in the cytoplasm are formed by the destruction and/or coalescence of fenestrae which permit red blood cells to penetrate into the space of Disse, resulting in collapsed sinusoids and impaired liver microcirculation[8, 23]. Alternatively, VEGFR1 TK signaling protects LSECs against APAP hepatotoxicity and minimizes liver microcirculatory disturbance to ensure blood supply of the regenerating liver. Expression of several pro-angiogenic growth factors, including basic fibroblast growth factor (bFGF) and transforming growth factor (TGF) β is up-regulated through VEGFR1 TK signaling[23]. Exogenous VEGF promotes the recovery from LSEC injury to APAP toxicity, which is associated with enhanced hepatic expression of VEGFR1 at later time points (36 h after APAP)[28]. Although VEGF/VEGFR1 pathway may regulate the functional integrity of LSECs[29], the mechanism of protective effect of VEGFR1 signaling on LSECs during APAP hepatotoxicity remains to be elucidated.

Moreover, during repair phase of APAP hepatotoxicity, VEGFR2 signaling enhances hepatic expression of CD31 and endothelial nitric oxide synthase (eNOS), which could contribute to the sinusoidal restoration[7, 21]. VEGFR1-TK signaling facilitates the restoration of LSEC from APAP hepatotoxicity through the maintenance of VEGFR2 expression on LSECs[23]. In the model of liver regeneration induced by partial hepatectomy, VEGFR2 activation is crucial for liver regeneration through neoangiogenesis[30]. Also, in liver regeneration following carbon tetrachloride toxicity, VEGFR2 signaling stimulates LSEC proliferation[31].

Roles of VEGFRs in liver repair through cellular cross-talk between parenchymal and non-parenchymal cells during APAP hepatotoxicity

During APAP hepatotoxicity, both VEGFR1 and VEGFR2 signaling promote hepatocyte proliferation as indicated by enhanced expression of proliferating cell nuclear antigen (PCNA), a marker of cellular proliferation[21, 23]. VEGF, tumor necrosis factor (TNF) α, hepatocyte growth factor (HGF), and other mediators have been implicated in promoting liver tissue regeneration after an APAP overdose[21, 23, 26].

Cellular cross-talk between LSECs and hepatocytes plays an important role in sinusoidal homeostasis and physiologic angiogenesis during liver regeneration[30, 32]. In liver regeneration following carbon tetrachloride toxicity, VEGFR1 activation elicits paracrine release of tissue specific growth factors (HGF and interleukin-6 (IL-6)) from LSECs, resulting in the proliferation of hepatocytes[31]. In the partial hepatectomy-induced liver regeneration, VEGFR2 signaling in LSECs facilitates angiogenesis through up-regulation of Id1 and secretion of HGF and Wnt2[30].

Contact between macrophages and hepatocytes also is crucial for liver repair after toxin-induced liver injury. Evidence suggests that M2 macrophages generate a variety of growth factors such as TGFβ, VEGF, and epidermal growth factor (EGF), which are key to angiogenesis, tissue regeneration, and repair[33]. We recently have shown that recruited macrophages promote liver repair after carbon tetrachloride hepatotoxicity through production of TNF, IL-6, and HGF[34].

Conclusion

In conclusion, VEGF/VEGFR signaling appears to be crucial for liver repair after APAP hepatotoxicity (Fig.2). The recruitment of macrophages in the injured areas through VEGFR1 signaling and enhanced VEGFR2 expression promote liver repair as indicated by restoration of the hepatic sinusoids and hepatocyte proliferation mediated by TNF and HGF. Highly selective VEGFR1 and VEGFR2 agonists may serve as novel therapeutic tools to aid in the repair of tissue damage from acute liver injury.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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