Platelet-activating factor in liver injury: A relational scope

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Abstract

The hepatocyte, the main cellular component of the liver, exhibits variable susceptibility to different types of injury induced by endogenous or exogenous factors. Hepatocellular dysfunction or death and regeneration are dependent upon the complicated interactions between numerous biologically active molecules. Platelet-activating factor (PAF) seems to play a pivotal role as the key mediator of liver injury in the clinical and experimental setting, as implied by the beneficial effects of its receptor antagonists. A comprehensive up-to-date overview of the specific functional and regulatory properties of PAF in conditions associated with liver injury is attempted in this review.

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Key words: PAF; PAF-R antagonists; Liver; Injury; Regeneration

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INTRODUCTION

Platelet-activating factor (PAF) (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent pro-inflammatory lipid mediator[1,2] presenting a very broad spectrum of biological activities, including allergic reactions, inflammatory conditions, cardiovascular and neuronal function, reproduction, ischemia-reperfusion (IR) organ injury, sepsis, shock and tumorigenesis, which are being continuously populated[3]. PAF is typically produced by several types of leukocytes (i.e. basophils, eosinophils, neutrophils, macrophages and monocytes), platelets, endothelial cells and many other types of stimulated cells and has been detected in virtually all biological fluids. PAF exerts its activity through binding to a specific receptor, PAF-R, a seven transmembrane, heterotrimeric G protein-linked membrane receptor[4]. PAF-R transduces pleiotropic functions such as cell motility, smooth muscle contraction, synthesis and release of mediators and cytokines[5]. It has also been speculated, supported by the observation that most of the synthesized PAF remains within the cell, that PAF possibly plays an intracellular role in addition to its function as an extracellular communication molecule[6]. Moreover, PAF may play a role in many pathophysiological processes as a cofactor, i.e. as a distinct component of a complex mediator network. PAF is enzymatically synthesized through two major routes, the so-called remodeling and de novo pathways, with the contribution of an acetyltransferase (PAF-AT) and is degraded by acetylhydrolases (PAF-AH) into its biologically inactive form, lyso-PAF[7]. The biochemical steps of biological PAF synthesis and degradation are schematically presented in Figure 1. PAF-AHs exhibit phospholipase A2 (PLA2)-like activity leading to hydrolysis of the m2 ester bond, reversing the acetyltransferase synthetic step and generating lyso-PAF and acetate. One plasma-type and two intracellular PAF-AH have been described and cloned[8]. Plasma PAF-AH is a monomeric enzyme synthesized and secreted by macrophages (and to a lesser extent in the liver) and associated with lipoproteins in plasma. Intracellular PAF-AHs are divided into types I and II, with type II PAF-AH showing a broader specificity and significant homology to the plasma-type enzyme. PAF-AHs obviously control, through elimination or inhibition, PAF activity in cases of excessive production and release of this potent mediator[9], although their precise role in normal and disease state is still poorly understood.

PAF has been suspected to play an important role in liver pathophysiology, particularly associated with inflammatory conditions. Its contribution as a mediator to the pathogenesis of liver injury in regenerating livers, through activation of multiple intermediate molecules or cofactors, has been elucidated in several experimental studies. In the liver, PAF is mainly produced and released by Kupffer cells facilitating communication and interaction between hepatic sinusoidal and parenchymal cells. The regulatory role of PAF in leukocyte recruitment, microvascular dysfunction and cytokine production associated with liver injury remains a main target of current research.

This review aims to present in a collective way the information available concerning the involvement of
PAF in various types of liver injury in order to reveal its crucial role in liver pathophysiology. The already reported effects of specific PAF-R antagonists on liver injury and regeneration are also mentioned.

**PAF AND LIVER INJURY**

**PAF and hepatic ischemia-reperfusion (IR) injury**

Hepatic ischemia-reperfusion (IR) injury is a common issue encountered in various clinical conditions, including systemic shock followed by hepatic failure, liver transplantation and liver resections or extensive hepatectomy due to trauma or cancer. Although hepatocellular injury does occur during the period of ischemia or hypoperfusion of the liver, there is increasing evidence that most of the hepatic cellular damage occurs during reperfusion of the ischemic liver, supporting the assumption that hepatic injury due to ischemia is accentuated after the re-establishment of oxygen flow.[10] Among the mechanisms accounting for this type of liver injury, which include oxygen-derived free radical formation, cellular energy depletion leading to cell membrane dysfunction[11], disruption of calcium homeostasis[12], activation of phospholipases[13], production and release of soluble mediators such as interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor (TNF)-α, neutrophil stimulation, chemotraction and adhesion to activated endothelial sites resulting in microvascular injury[14-16], PAF is thought to play a major role as a mediator of the inflammatory events following hepatic IR[17]. Several experimental animal models have been used to investigate the extent of cellular disruption and the protective mechanisms involved in liver IR injury in association with PAF activity and the potentially beneficial effects of potent PAF-R antagonists. The variability of these models remains a major issue limiting the reliable assessment and comparison of their results. Studies conducted as yet can be schematically divided into two groups: in situ and isolated hepatic IR studies. Table 1 summarizes the features of experimental studies using PAF-R antagonists.

Liver function following hepatic IR has been initially assessed in studies using an isolated liver perfusion apparatus. In an early study[18] comparing cold liver preservation in the Eurocollins and the University of Wisconsin (UW)-lactobionate solutions, the efficacy of pretreatment with SRI-63441, a potent PAF-R antagonist, was also evaluated. Livers isolated from pretreated rats and stored in the UW solution for 24 h produced significantly more bile than livers preserved in the UW solution alone. Although the differences in other parameters of liver function evaluated in this study did not reach statistical significance, the observed beneficial effect was attributed to PAF antagonism, supporting the assumption that PAF was implicated in cold ischemic liver injury. The role of PAF and its antagonist CV-6209 in cold ischemic liver injury of similar duration has also been investigated[19]. After 24 h of cold preservation, bile flow was significantly higher in all CV-6209-treated groups at two hours of reperfusion and at specific time points with a dose-dependent recovery outcome, which also correlated with significantly lower aspartate aminotransferase (AST) levels. A tendency toward lower liver weight increase and lower perfusion pressures was also noted. Using the same isolated liver perfusion system, an experimental series of warm liver ischemia revealed better recovery of bile flow in groups which received higher doses of CV-6209 over a specific threshold of antagonism. In addition, AST level increase was significantly lower within certain dosage limits and a tendency toward lower perfusion pressures was observed. These results suggested that PAF was possibly implicated in both warm and cold ischemic liver injuries, which might be effectively diminished by a potent receptor antagonist. SRI-63441 has also been used in another series of isolated liver perfusion experimental studies[20] aiming to evaluate the pharmacologic modulation of warm postischemic hepatic function. Pretreatment with this PAF-R antagonist before the induction of in situ total hepatic ischemia resulted in significantly increased bile production, decreased perfusate transaminase levels and higher tissue adenosine triphosphate (ATP) content compared to ischemic non-treated controls. The degree of hepatocellular vacuolization and sinusoidal endothelial disruption due to ischemic injury revealed by electron microscopy was also less severe in the pretreatment group. These findings confirmed the dose-dependent protective effect of this antagonist against warm IR injury, thus implicating PAF as a key mediator of this type of liver injury. The direct effect of PAF on ischemic liver injury has been investigated in an ex situ isolated rat liver perfusion system[21], in order to avoid the interference of other factors. Administration of high doses of PAF, expected to result in shock and death in vivo, did not exacerbate hepatic ischemic injury in this model after 30 or 60 min of ischemia. This finding suggested that PAF, unlike its in vivo action, neither causes hepatocellular injury nor enhances ischemic liver injury directly, supporting the hypothesis that PAF exerts its effects through other factors and substantially acts as a key mediator of ischemic liver injury. However, in another similar study, administration of increasing doses of PAF significantly decreased liver tissue ATP levels and oxygen consumption and significantly increased alanine aminotransferase (ALT) and purine nucleoside phosphorylase (PNP) activity in the effluent perfusate compared to control values[22]. The noted disruption of
parenchymal cells in association with the unaltered PNP/ALT ratio, used as a relative indicator of non-parenchymal cell injury, suggested that PAF might lead to functional impairment of parenchymal hepatic cells, i.e. hepatocytes, even in the absence of microcirculatory disturbance secondary to the interaction between circulating leukocytes and endothelial cells.

Experimental studies of in situ hepatic IR have consistently used models of total, with or without splanchic congestion, or partial liver ischemia. Partial liver ischemia by clamping or ligation of the hilar area of the left lateral and median rat liver lobes, thereby avoiding intestinal congestion, has been extensively applied in several warm hepatic IR studies. Demonstration of the protective effects of CV-6209, in terms of improved bile flow and earlier recovery of rat liver ATP levels and energy charge after 60 min of ischemia, as well as determination of the optimal timing and dosing pattern were attained in a leading study conducted by Wang et al. The contribution of PAF to the inflammatory consequences of hepatic IR injury, especially during the late-phase hepatic reperfusion, has been evaluated by measurement of hepatic PAF levels and intraperitoneal administration of the PAF-R antagonist WEB-2170. Treatment with WEB-2170 resulted in a significant decrease of plasma ALT levels and polymorphonuclear (PMN) leukocytes infiltration in liver tissue in association with impaired hepatocellular necrosis compared to the control group. Additional pharmacological interventions provided evidence that interaction between Kupffer cells and oxygen-derived free radicals led to local hepatic generation of PAF after IR. A survival study using a partial hepatic IR injury model demonstrated that pretreatment with the PAF-R antagonist BN-52021 resulted in a statistically significant reduction of warm liver injury in terms of improved seven-day cumulative survival rates and histological evidence of minor hepatocellular necrosis on the first postoperative day and revealed a dose-dependent protective effect.

Pre-ischemic administration of BN-52021 in a similar hepatic IR model resulted in a significant reduction of hepatocellular loss of cytoplasmatic ALT and intramitochondrial glutamate dehydrogenase (GLDH) into the plasma. In this study, significantly reduced endothelial enzyme leaking (PNP), increased bile flow and liver tissue ATP content and moderately decreased malondialdehyde (MDA) levels in the pretreated reperfused livers were observed, suggesting the implication of PAF in both structural and metabolic changes induced by hepatic IR injury. Microcirculatory disruption due to hepatic IR has also been investigated by measurement of hepatic PAF levels and photometry, respectively. It was therefore assumed that the involvement of PAF might be secondary in the pathogenesis of reperfusion injury or bypassed by other major contributors, such as leukocytes in this instance. Nevertheless, another contemporary study evaluating the role of PAF in microcirculatory disturbance in a similar experimental model demonstrated that pretreatment with BN-52021 resulted in significant improvement of left lobular erythrocyte flux recovery, reaching nearly preischemic control values after 10 min of reperfusion. In addition, PNP leakage from hepatic vascular endothelium into plasma as a result of reperfusion was significantly attenuated by administration of BN-52021. A statistically significant reduction of posts ischemic hepatocellular ALT loss, accompanied by enhanced bile production and increased tissue ATP levels as well as by suppressed neutrophil infiltration in liver tissue, as indicated by reduced myeloperoxidase (MPO) activity, were also observed in the pretreatment group, suggesting that PAF might be implicated in IR-induced microcirculatory disturbance.

The emerging controversy between these studies seems to have been resolved by a recent experimental evaluation of the effects of PAF and a thromboxane (Tx)-A2 analogue.

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**Table 1  Experimental studies of IR liver injury evaluating the role of PAF through its antagonism**

| Ischemia | Reperfusion |
| --- | --- |
| Type of ischemia | Splanchnic congestion | Duration | PAF-R antagonist | Type of reperfusion | Duration | Reference |
| is, T, W | No | 120 min | TCV-309 | is | 30 min | [34] |
| is, T, W | No | 120 min | TCV-309 | is | 30 min | (W), 60 min | [32] |
| is, T, W | Yes | 20 min | E8880 | is | 30 min | (C) | [31] |
| is, T, W | Yes | 45 min | BB-882 (lexipafant) | is | 45 min | [35] |
| is, T, W | NA | 120 min | WEB-2170 | is | 6, 24 h | [37] |
| is, T, W | NA | 60 min | BN-52021 | is | 60 min | | [38] |
| is, P, W | No | 60 min | WEB-2170 | is | 1, 3, 6, 24 h | [24] |
| is, P, W | No | 30, 45 min | WEB-2170 | is | 1 h | [28] |
| is, P, W | No | 60 min | BN-52021 | is | 30 min | | [27] |
| is, P, W | No | 60 min | BN-52021 | is | 30 min | | [29] |
| is, P, W | No | 75 min | BN-52021 | is | 24 h | s 7 d | [26] |
| is, P, W | No | 30, 60, 90 min | CV-6209 | is | 15, 30, 60, 120 min | [23] |
| is, T, W+C | NA | 90 min (W), 24 h (C) | CV-6209 | Is | 60 min (W), 120 min (C) | [19] |
| is, T, W | NA | 90 min | SRI-63441 | Is | 90 min | | [20] |
| is, T, C | NA | 24 h | SRI-63441 | Is | 90 min | | [18] |

is: In situ; Is: Isolated; T: Total; P: Partial; W: Warm; C: Cold; NA: Not applicable; s: Survival; d: Days.
on hepatic vascular resistance distribution and liver weight in an isolated perfused animal model\textsuperscript{[39]}. PAF was shown to be a potent vasoconstrictor causing a dose-dependent increase of total hepatic vascular resistance. Furthermore, it was demonstrated that PAF increases predominantly pre-sinusoidal over post-sinusoidal resistance and influences liver weight, causing liver weight loss at low concentrations and liver weight gain at high concentrations. These findings support the presumption that PAF is involved in the induction of hepatic microcirculatory disturbance in conditions such as IR injury.

In many recent studies, total hepatic ischemia has been applied using the Pringle’s maneuver, i.e. by ligation or cross-clamping of the portal triad - hepatic artery, portal vein and bile duct. The effects of pretreatment with the PAF-R antagonist E5880 on hepatic and systemic metabolism have been investigated after a relatively short period of \textit{in situ} total warm hepatic ischemia followed by a similarly short period of reperfusion, without avoiding intestinal congestion\textsuperscript{[30]}. In the pretreatment group, hepatic tissue ATP and energy charge levels were significantly increased while AMP levels were significantly decreased after 30 min of reperfusion compared to non-pretreated animals. However, no statistically significant difference was noted in parameters reflecting hepatic mitochondrial function, such as the arterial ketone body ratio (AKBR - ratio of acetoacetate to 3-hydroxybutyrate) and arterial ammonia levels, or those indicating systemic changes, such as arterial blood gas values and pyruvate and lactate levels. These data suggested that PAF exerts local effects during liver IR injury, attenuated by its antagonism, without mediating systemic metabolic changes. The effects of the specific PAF-R antagonist TCV-309, alone or in combination with a prostaglandin I\textsubscript{2} analogue, on total warm hepatic IR injury were evaluated in a large study\textsuperscript{[32]}. After two hours of \textit{in situ} hepatic ischemia followed by one hour of reperfusion, improvement of bile flow rate, suggesting restoration of hepatic ATP levels, a significant decrease of tissue glutathione disulfide/glutathione (GSSG/GSH) ratio and less severe midzonal liver injury and congestion were observed in groups treated with TCV-309, indicating preservation of hepatic function and attenuation of oxidative stress. Despite a significant increase in portal endotoxin levels in pretreated animals, 30 d survival rates were higher compared to non-pretreated groups. These results suggested that PAF was generated in the liver during warm IR leading to liver injury. Using the same PAF-R antagonist, a similar study focusing on cytokine production and neutrophil activation induced by PAF after hepatic IR was conducted\textsuperscript{[36]}. Total hepatic ischemia was produced while intestinal congestion was avoided using an extracorporeal portosystemic shunt. During the reperfusion period, an increased 30-d survival rate, decreased plasma AST levels, significant suppression of plasma TNF-\textalpha and CINC (cytokine-induced neutrophil chemoattractant) levels, less severe hepatic necrosis, markedly decreased neutrophil accumulation and significantly lower O\textsubscript{2} production in liver tissue were noted in the pretreatment group compared to the control group. Inhibition of TNF-\textalpha and CINC production by the specific PAF-R antagonist TCV-309 strongly suggested that PAF functions on hepatic IR injury were exerted through either direct neutrophil activation or cytokine induction. The role of this specific PAF-R antagonist in hepatic IR injury has been further evaluated in a recent survival study of total hepatic ischemia\textsuperscript{[34]}. Pretreatment with TCV-309 significantly suppressed the increase in plasma levels of AST and endothelin (ET)-1, a potent vasoconstrictor derived from vascular endothelium and hepatic sinusoidal endothelial cells which leads to hepatic microvascular injury, at all selected time points after reperfusion. In addition, at 6 h after reperfusion, hepatocellular necrosis and neutrophil accumulation in midzonal and pericentral areas of the hepatic lobule were less prominent in TCV-309-treated group. Similarly, survival rates demonstrated a statistically significant improvement in TCV-309-treated animals compared to controls. The complicated interaction between PAF and ET-1 in hepatic IR injury evaluated in this study supported the hypothesis that PAF-R antagonism might exert a potential protective effect against this type of injury via indirect modulation of plasma ET-1 levels. A most recent study of total hepatic IR\textsuperscript{[35]} showed that pretreatment with the PAF-R antagonist BB-882 (lexipafant) resulted in a statistically significant decrease of liver damage scores\textsuperscript{[60]}, the mean hepatic MDA levels (sensitive indicator of lipid peroxidation) and serum AST, ALT and lactate dehydrogenase (LDH) levels. These beneficial effects were attributed to a reduction in the inflammatory reaction and oxygen radical synthesis induced by PAF after hepatic IR injury. In order to confirm the putative etiologic relation between PAF, xanthine oxidase, reactive oxidants and leukocytes in the pathogenesis of liver injury, a modified hemorrhagic shock-resuscitation rat model based on Wigger’s isobaric principle has been used\textsuperscript{[57]}. In this unique study, inhibition of PAF-R binding by WEB-2170 significantly attenuated leukocyte accumulation in the pericentral areas of the hepatic lobule, both at the early inflammatory response time point and at 24 h end point, as demonstrated by total hepatic MPO activity and histochemical examination. Significantly less severe hepatic injury, evident by plasma AST and alcohol dehydrogenase (ADH) levels and histological grading of hepatocellular necrosis, was also observed in the WEB-2170-treated group, although there was no decrease in the generation of hepatic oxidant stress, as shown by hepatic reduced GSH and oxidized GSSG levels. These results, in combination with those yielded from the administration of other potentially protective factors, established an apparent etiologic relation between PAF and the aforementioned components of hepatic injury \textit{in vivo}. In another experimental model of hemorrhagic shock, causing whole-body IR, the effects of PAF on calcium homeostasis were investigated\textsuperscript{[59]}. PAF is known to use voltage- and receptor-gated Ca\textsuperscript{2+} influx to exert its actions in a variety of cell types\textsuperscript{[59]}. In this model, hepatocellular Ca\textsuperscript{2+} uptake, initial rate of Ca\textsuperscript{2+} influx and membrane Ca\textsuperscript{2+} flux, determined using specific incubation techniques\textsuperscript{[59]}, were significantly increased in the shock group compared to sham-operated animals. Administration of the specific PAF-R antagonist BN-52021 in the shock group significantly reduced Ca\textsuperscript{2+} uptake, without influencing other parameters of calcium homeostasis, and largely prevented hepatocyte lipid peroxidation, determined fluorometrically\textsuperscript{[60]}. These results suggested that PAF may play a pivotal
role in promoting hepatocyte Ca\(^{2+}\) overload during hemorrhagic shock, which in turn accentuates hepatic oxidative injury.

**PAF and liver transplantation**

In the field of clinical liver transplantation, liver injury due to cold ischemia followed by warm reperfusion, referred to as preservation-reperfusion injury (PRI) of the graft, remains one of the most critical issues affecting postoperative graft viability and ultimate survival of the recipient. It has been well established that hypothermic ischemia mainly causes sinusoidal lining cell injury which becomes fully manifest on reperfusion, leading to deterioration of the microcirculation\(^{\text{[41]}}\). Although the exact mechanisms have not yet been fully elucidated, PAF has been proposed as a major mediator involved in the pathogenesis of PRI and several studies have attempted to clarify the potentially protective role of PAF-R antagonists, as shown in Table 2.

A clinical study of 17 patients who underwent orthotopic liver transplantation (OLT)\(^{\text{[42]}}\) revealed that peripheral blood PAF levels were not significantly altered during the immediate reperfusion period or during the acute rejection episodes, confirming that successful OLT normalizes blood PAF levels in cirrhotic decompensated patients and that PAF levels were probably unrelated to the onset of acute graft rejection. On the other hand, in another clinical study, PAF levels were increased after portal venous reperfusion, except for one case where veno-venous bypass was applied, suggesting that PAF was probably involved in postoperative liver graft dysfunction\(^{\text{[43]}}\).

Experimental studies evaluating the role of PAF in liver transplantation through its antagonism have consistently used porcine or rat models. In a rat OLT model, administration of BN-52021 in the preservation solution and to the recipient prior to liver graft implantation\(^{\text{[44]}}\) resulted in significant reduction of serum LDH and PNP levels and significant improvement of hepatic bile production after reperfusion in the treated group compared to untreated controls. A tendency toward suppressed oxygen free radical generation, as indicated by attenuation of serum conjugated dienes (CD) levels, and liver tissue-associated MPO activity was also noted in the treatment group, although the differences were not statistically significant. The potentially protective effect of the potent PAF-R antagonist E5880 on PRI of the liver graft has been evaluated in another OLT study\(^{\text{[45]}}\). Survival rates after 12 h of reperfusion, AKBR, an accurate marker of liver graft metabolic function, and white blood cell (WBC) count were significantly higher, whereas AST and ALT levels at 4 h and lactate levels were lower in the treatment group compared to controls. Histological examination revealed only occasional presence of inflammatory cells, mainly PMNs, in the sinusoids of the treatment group. These results confirmed the implication of PAF in PRI as well as the protective effect of its antagonism. A similar study demonstrated that treatment with WEB-2170 resulted in suppressed, though not statistically significant, postoperative elevation of hepatocellular enzymes (AST, ALT) and bilirubin levels in hepatic venous blood samples and in normalization of local tissue oxygen tension after 6 h of reperfusion, as determined by the use of a multiwire oxygen electrode on liver surface\(^{\text{[46]}}\).

These data suggested that PAF antagonism attenuated hepatocellular injury and prevented the detrimental shifts of local tissue oxygen tension which reflects the integrity of hepatic microcirculation. The role of PAF as a mediator involved in the regulation of leukocyte adhesion after OLT has been evaluated through administration of the PAF-R antagonist WEB-2086\(^{\text{[47]}}\). In the treatment group, the temporary leukocyte adhesion to sinusoidal endothelial cell lining in the periportal region, determined by the method of intravital fluorescence microscopy, was significantly reduced in the early reperfusion period followed by a similar reduction of permanent leukocyte adhesion in the late one. Comparable changes in midzonal and pericentral areas were also noted. Suppression of leukocyte adhesion by this PAF-R antagonist was further reflected by an increase in peripheral blood WBC count during the early reperfusion period. These results suggested that PAF antagonism attenuated the inflammatory response in hepatic sinusoids, thus contributing to the preservation of microcirculation, whereas no protective effect on hepatocellular integrity was noted. The effects of E5880 on PRI of the liver graft in porcine OLT were further assessed in a survival study\(^{\text{[48]}}\), in which postoperative survival of more than 12 h was significantly higher in the PAF-R antagonist treatment group. Treated animals demonstrated a slight but significant increase in WBC count at 2 h after reperfusion and at 4 h AST, ALT and lactate levels were significantly lower compared to the control group. In addition, the AKBR was significantly increased at 12 h after reperfusion, indicating better recovery of liver graft function. Histological examination by light and electron microscopy revealed less severe sinusoidal endothelial cell damage in the treatment group. Similar results were obtained in another study in which prolonged warm hepatic ischemia in non-heart-beating donors was followed by cold preservation and reperfusion\(^{\text{[49]}}\). Improved survival rate at 12 h after OLT, suppression of serum AST and lactate levels, attenuation of hepatocellular degeneration and significantly better energy charge recovery were noted in animals treated with E5880 compared to controls. Although most parameters

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Table 2 PAF and liver transplantation

| Type of study | PAF-R antagonist used | Alterations in biochemical parameters of liver graft function (serum) | Reference |
|---------------|-----------------------|---------------------------------------------------------------|-----------|
| Clinical      |                       |                                                               |           |
|               |                       | ↑ AKBR, ↓ AST, ↓ ALT, ↓ lactate                               |           |
|               |                       | NS                                                            |           |
|               |                       | ↓ LDH                                                         |           |
|               |                       |                                                               |           |
| Experimental  | BN-52021              | ↓ LDH                                                         | [45]      |
|               | WEB-2170              | NS                                                            | [47]      |
|               | WEB-2086              | ↓ LDH                                                         | [48]      |
|               | E5880                 | ↑ AKBR, ↓ AST, ↓ ALT, ↓ lactate                               | [46]      |
|               | E5880                 | ↓ AST, ↓ lactate                                              | [50]      |

1 Only statistically significant alterations are included in the table above; 2 Not determined; NS = Not significant; LDH = Lactate dehydrogenase; AKBR = Arterial ketone body ratio; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase.
did not reach statistical significance, this was partly attributed to the small number of treated animals.

**PAF and acute liver injury**

Acute hepatic dysfunction, or even failure, occurs frequently in the clinical setting and is usually refractory to currently available treatment options. The pathogenesis of hepatocellular damage has been well characterized in several experimental studies of acute liver injury induced by lipopolysaccharide (LPS) compounds, chemical and pharmacological factors, bile duct ligation or hepatectomy (Table 3).

In an early study of endotoxin-induced liver injury by intravenous infusion of heat-killed *Propionibacterium acnes* and LPS, treatment of experimental animals with a PAF-R antagonist attenuated liver tissue damage, as indicated by the reduction of focal liver necrosis in histological examination. In addition, liver adherent cells isolated from control and endotoxin-treated mice were capable of producing PAF upon stimulation with A23187 calcium ionophore. These results suggested that the produced PAF by liver adherent cells could be involved in the induction of liver injury during endotoxemia. The effects of endotoxin and PAF in rats with secondary biliary cirrhosis were also evaluated and it was suggested that the hemodynamic changes observed in cirrhosis might be aggravated during acute infections. LPS administration significantly increased heart rate and total peripheral vascular resistance and reduced cardiac index, portal pressure and renal blood flow in cirrhotic animals compared to controls. PAF infusion resulted in similar changes in the experimental group, with the exception of a sharp rise in portal pressure owing to an increase in portal territory vascular resistance. Treatment with the specific PAF-R antagonists WEB-2170 or BN-52021 largely prevented LPS-induced renal vasoconstriction and the former also restored cardiac index and peripheral resistance to near normal levels. The complicated interactions between LPS, PAF and TNF-α have been further elucidated. Administration of either LPS or PAF resulted in elevated serum TNF-α levels and TNF-α mRNA production in the intestine and the liver in response to LPS was only partially inhibited by the PAF-R antagonist WEB-2086. These results suggested that LPS induces TNF-α production via both PAF-dependent and PAF-independent pathways and that these major inflammatory mediators interact through a positive feedback mechanism. LPS-associated activation and PAF-induced biochemical response of hepatic macrophages and endothelial cells have also been investigated. PAF administration resulted in a rapid and transient increase in intracellular calcium content in both cell types whereas a rapid decrease in intracellular pH of hepatic macrophages only, attenuated by the PAF-R antagonist triazolam, was also noted. In addition, LPS caused a significant increase in the number of PAF-binding sites/cell and the equilibrium dissociation constant (Kd) for PAF in endothelial cells. These results revealed that the biochemical responses of these two major hepatic cell types to PAF during endotoxemia are distinct. In a unique model of LPS-induced liver injury, based on evidence that intestinal IR increases portal and systemic plasma endotoxin levels, the role of Kupffer cells in mediating leukocyte recruitment, impaired sinusoidal perfusion and liver tissue hypoxia via complicated interaction with PAF and TNF-α was investigated. In this study, treatment with WEB-2086 effectively attenuated LPS-induced leukostasis in all segments of hepatic microvasculature, the number of nonperfused sinusoids and elevation in plasma ALT and TNF-α levels. Furthermore, the increased autofluorescence of pyridine nucleotide [NAD(P)H], an indicator of mitochondrial oxygen consumption and redox status, was significantly suppressed in both midzonal and pericentral areas and expression of P-selectin, an endothelial cell adhesion molecule, was depleted by this antagonist in the liver and the small intestine. It was also reported that excessive nitric oxide (NO) generation by hepatic cells, especially Kupffer cells, in response to LPS and inflammatory mediators, such as PAF, contributes to the pathogenesis of liver injury. In a model of rat liver-focused endotoxemia, pretreatment with specific PAF-R antagonists (BN-50739 or WEB-2170) reduced serum ALT and inducible nitric oxide synthase (iNOS) mRNA levels in the whole intact liver. In addition, pretreatment of cultured Kupffer cells with either of these antagonists resulted in significant inhibition of both LPS and PAF-induced iNOS mRNA and protein synthesis. Previous studies have shown that iNOS gene activation in macrophages by LPS depends on the transcription factor nuclear factor-kappa B (NF-κB), whose activation results in degradation of the α and β subunits of its inhibitor (IkB). It was shown that PAF-R antagonists attenuated the translocation of the active p65 subunit of NF-κB into the nucleus. A concomitant suppression of LPS-dependent degradation of the inhibitory protein subunit IkBα and intracellular calcium content of Kupffer cells was also noted. These results confirmed the protective effects of PAF antagonism against NO-mediated inflammatory liver tissue

| Method       | PAF levels | PAF-R antagonist used | Reference |
|--------------|------------|-----------------------|-----------|
| LPS/P.acnes  | ↑ serum    | BN-50201, WEB-2170    | [51]      |
| LPS/PAF      | ↑ serum    | WEB-2086              | [52]      |
| LPS          | ↑ serum    | triazolam             | [53]      |
| D-GalN/LPS   | ↑ serum    | WEB-2086              | [54]      |
| APAP         | ↑ (serum)  | BN-52021              | [55]      |
| BDL          | ↑ (serum)  | WEB-2086              | [56]      |
| 70% PH       | ↑ (serum)  | TCV-309               | [57]      |

1 Not determined; LPS = Lipopolysaccharide; D-GalN = D-galactosamine; ANIT = Alpha-naphthylisothiocyanate; APAP = Acetaminophen; BDL = Bile duct ligation; PH = Partial hepatectomy.
injury during endotoxemia. In contrast to the results of the aforementioned studies, pretreatment of experimental animals with WEB-2086 one hour before intravenous LPS administration failed to inhibit LPS-induced sinusoidal neutrophil infiltration, evaluated by histological examination of hepatocellular necrosis and indicated by elevated plasma ALT activity, and did not significantly alter thrombocytopenia or plasma fibrinogen levels. However, it has been speculated that the conflicting results might derive from variations related to the experimental models used in terms of the route of LPS administration, the degree of hepatocellular injury and temporal differences in the development of liver injury and histological evaluation. The role of PAF in alcoholic hepatitis in association with LPS administration has been studied by Murohisa et al. Chronic ethanol administration remarkably sensitized experimental animals to the effects of LPS and severe hepatocellular injury associated with significantly elevated serum ALT, TNF-α, and IL-8 levels was observed. Histological findings of liver injury included hepatocyte apoptosis and necrosis with marked neutrophil infiltration and enhanced Fas-receptor expression on hepatocytes and Fas-ligand expression on infiltrating neutrophils. Pretreatment with TCV-309 attenuated hepatocellular apoptosis and necrosis, neutrophil infiltration, Fas-receptor and Fas-ligand expression and decreased serum TNF-α levels. These protective effects suggested that PAF acts as a key mediator of endotoxin-induced liver injury in alcoholic hepatitis and possibly regulates Fas-receptor expression on hepatocytes via TNF-α, thus facilitating neutrophil accumulation and hepatocellular apoptosis. In an experimental model of liver-focused endotoxemia, LPS administration resulted in a significant increase in plasma-type PAF-AH mRNA and protein expression, particularly in Kupffer cells. A similar increase was noted in both circulating leukocytes and peritoneal macrophages as well as in all tissues examined. Administration of the PAF-R antagonists BN-50739 and WEB-2170 significantly inhibited the increase in PAF-AH activity in response to LPS, suggesting that PAF-R is probably involved in the upregulation of this enzyme, thereby minimizing the unfavorable hepatic and systemic effects of PAF-mediated injury. Two independent studies on D-galactosamine (D-GalN)/LPS-induced rat liver injury confirmed the protective effects of the potent PAF-R antagonist WEB-2086. In the first study, a significant suppression of elevated transaminase levels and less severe histological changes were noted in the WEB-2086-treated animals compared to controls. Furthermore, PAF-R antagonism resulted in a significant attenuation of D-GalN/LPS-induced elevation of MDA and MPO levels and prevented the decrease of superoxide dismutase (SOD) in the liver tissue of WEB-2086-treated animals. In the second study, both in vivo and in vitro effects of WEB-2086 were investigated. The degree of hepatic injury and the levels of transaminases were significantly decreased in WEB-2086-treated animals after D-GalN/LPS administration and a significantly reduced number of infiltrating neutrophils was observed compared to the untreated group. In vitro evaluation revealed that the enhanced neutrophil adhesion to hepatic endothelial cells, either by stimulation with the sera collected from D-GalN/LPS treated animals or due to a direct effect of LPS, TNF-α or PAF, was also attenuated by WEB-2086 in a dose-dependent manner. These results strongly suggested a crucial role for PAF and PAF-R antagonists in this specific type of liver injury.

In an experimental study of acute, chemical-induced cholestasis by administration of the cholangiotoxic alpha-naphthylisothiocyanate (ANIT), the role of PAF was investigated through pre- and post-treatment of study animals with WEB-2086. ANIT-induced hepatotoxicity was confirmed by histological evaluation of periportal inflammation with marked neutrophil infiltration and biochemical parameters indicative of cholestasis. Treatment of the experimental group with WEB-2086 did not attenuate ANIT-induced liver injury as shown by increased plasma ALT and γ-glutamyl transferase (γGT) activities, or cholestasis, as indicated by increased plasma bilirubin concentration. These results suggested that soluble mediators other than PAF might be primarily involved in neutrophil-dependent liver injury caused by this hepatotoxic agent. In a most recent study evaluating the role of PAF in acetaminophen (APAP)-induced liver injury and regeneration, blood PAF levels and serum PAF-AH activity were measured in association with a biphasic pattern of APAP hepatotoxicity, as indicated by histological findings and three independent indices of liver regeneration. Blood levels of free PAF were significantly elevated during the first wave of liver injury and peak levels temporally coincided with the maximal levels of AST, ALT and ALP activity. Serum PAF-AH activity showed different time course from that of PAF and hepatic enzymes following the biphasic pattern of liver regeneration, with the first rise occurring before the elevation of PAF levels and the second rise coinciding with the second wave of regeneration, probably in response to the prior high levels of PAF. These results suggested that PAF plays a major role in the initial priming of the liver regeneration process, possibly acting as an intracellular mediator, and that PAF-AH seems to eliminate PAF-mediated liver injury mainly during the late phase of APAP-induced hepatotoxicity. The protective effects of the PAF-R antagonist BN-52021 on APAP-induced acute liver injury have been recently reported in a serial study conducted by Grypioti et al. Intraperitoneal administration of this PAF-R antagonist resulted in a significant decrease of serum hepatic AST, ALT (indicative of necrosis) and alkaline phosphatase (ALP) (indicative of cholestasis) activities at several time points, accompanied by a remarkable elevation of serum PAF-AH activity at all time points compared to the control group. In addition, APAP-induced hepatocellular necrosis and apoptosis were significantly attenuated in BN-52021-treated animals. These results suggested that PAF might play a major role in liver injury and regeneration following acute APAP intoxication, underlying the beneficial effects of PAF-R antagonism. In an experimental model of obstructive jaundice by bile duct ligation, a significant increase of liver tissue and plasma PAF levels was observed. In another rat model of experimental obstructive jaundice of the same duration, PAF levels were significantly increased in liver tissue only, whereas no changes in plasma PAF levels were observed. In this study, endotoxin was detected in portal
blood from jaundiced animals and endotoxin antagonism by neomycin plus polymyxin B significantly reduced local hepatic PAF levels. Moreover, isolated Kupffer cells from jaundiced rats demonstrated higher levels of cell-associated PAF compared to other liver cell types and spontaneous release of PAF by these resident hepatic macrophages was significantly increased compared to controls, supporting their putative role as the major source of hepatic PAF production. Pretreatment of isolated Kupffer cells with polymyxin B selectively prevented LPS-induced PAF production, implying that the interaction of Kupffer cells with portal endotoxin constitutes a potential mechanism that triggers local hepatic PAF synthesis. Treatment with PAF-R antagonists (BN-52021 or WEB-2170) partially prevented the increase in tissue levels of eicosanoids (TXB₂, 6-keto-PGF₁α), lipid peroxidation, as was prominent by reduced MDA production, and liver injury, evidenced by reduced plasma ALT levels. The effects of recombiant PAF-AH on tissue damage and antioxidant response have been recently evaluated in an experimental model of obstructive jaundice[69]. On the seventh postoperative day, AST, ALT, γGT, ALP, TNF-α and IL-6 levels were significantly lower in the PAF-AH treatment group compared to rats with obstructive jaundice which did not receive PAF-AH treatment, although still significantly higher compared to controls. Portal inflammation was also less severe and increased levels of antioxidant enzymes were noted in the treatment group, suggesting a protective effect of PAF-AH through PAF degradation.

Postoperative acute liver injury remains one of the most serious complications of extended heptectomy and significantly influences postoperative morbidity and mortality. The implication of PAF in this type of injury was investigated in a rat model of 70% partial hepatectomy (PH)[70]. Post-PH, a significant rise in serum TNF-α, IL-6 and prostaglandin E₂ (PGE₂) levels was observed at 1, 3 and 6 h, respectively. No significant increase in portal venous endotoxin levels was noted. As early as 1 h post-PH, a significantly increased expression of PAF-R mRNA in Kupffer cells was noted in the group of heptectomized rats compared to sham-operated ones. These data suggested that PAF possibly stimulated Kupffer cells leading to upregulation of PAF-R and induced acute liver injury via the release of TNF-α and IL-6 from these cells, which was subsequently suppressed by enhanced PGE₂ production. The role of PAF in LPS-induced liver injury with regenerating livers post-PH has also been investigated[71]. Treatment of experimental animals with TCV-309 before or after LPS administration resulted in significantly higher survival rates, effective suppression of plasma ALT and CINC levels and reduced liver tissue necrosis with infiltration of CINC-positive neutrophils, compared to the saline treated controls. Furthermore, attenuation of continuous CINC mRNA upregulation and NF-κB activation in the regenerating livers by TCV-309 confirmed the crucial role of PAF in LPS-induced liver injury, possibly via neutrophil accumulation and activation.

**PAF and chronic liver injury**

Liver cirrhosis is a terminal pathologic state induced by several biological and chemical agents capable of producing chronic liver injury. Irrespective of the exact mechanisms by which etiologic factors induce liver injury, they all trigger a common final pathway that leads to excessive fibrosis and nodular regeneration of the viable liver tissue[72,74]. PAF has been implicated as a mediator of the pathologic, metabolic and hemodynamic changes observed in cirrhosis both in humans and experimental animal models. Table 4 presents clinical and experimental studies on chronic liver injury in association with PAF synthesis and antagonism.

In an early clinical report, increased PAF levels were observed in serum and ascitic fluid from decompensated cirrhotic patients, while compensated cirrhotics presented lower values but still higher than controls. In addition, serum PAF-AH activity was similar in all groups, supporting the conception of an enhanced production rather than a decreased degradation of PAF in cirrhotic patients[75]. Laffi et al further investigated alterations of PMN leukocyte function in nonalcoholic cirrhosis[76], based on well-established evidence of defective PMN function in patients with cirrhosis of alcoholic etiology[77]. PMN leukocytes from cirrhotic patients demonstrated in vitro a significantly reduced capacity to produce superoxide anion, determined by the reduction of cytochrome c, and a significant suppression of PAF synthesis, measured by [3H]acetate incorporation into PAF and mass-spectrometric analysis, in response to physiological (opsonized zymosan) and nonphysiological (calcium ionophore, A23187) stimuli. Impaired PAF synthesis was attributed to defective PLA₂ activity implicated in the remodeling pathway. In addition, it was shown that most of synthesized PAF from stimulated PMNs remained cell-associated rather than being secreted, supporting the hypothesis that PAF may also function as an intracellular second messenger[78]. However, it has been argued that these in vitro results need to be confirmed in an in vivo model[79], since elevated serum PAF levels in cirrhotic patients have been previously reported. In a clinical study, patients with chronic cholestasis, especially those with end-stage primary or secondary biliary cirrhosis or cholangiocarcinoma, demonstrated increased serum PAF-AH activity[80]. Normalization of liver function after liver transplantation

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### Table 4: PAF and chronic liver injury (cirrhosis)

| Type of study | Method of cirrhosis induction | PAF levels | PAF-R antagonist used | Reference |
|---------------|-------------------------------|------------|-----------------------|-----------|
| Clinical      | NA                            | ↑          | -                     | [76]      |
|               | NA                            | ↓          | -                     | [77]      |
|               | NA                            | NS         | -                     | [81]      |
|               | NA                            | ↑          | -                     | [82]      |
| Experimental  | CCl₄+                         | ↑          | BN-52021              | [84]      |
|               | phenobarbital                 | ↑          | -                     | [86]      |
|               | TAA                           | ↑          | -                     | [87]      |
|               | CCl₁+                         | ↑          | BN-52021              | [88]      |

1 Not determined; NA = Not applicable; NS = Not significant; CCl = Carbon tetrachloride; TAA = Thioacetamide.
was accompanied by a reduction to normal or near normal serum PAF-AH levels, suggesting that the liver plays an important role in the regulation of this degrading enzyme and, consequently, PAF. Moreover, it was shown that circulating levels of PAF are not a determinant of PAF-AH release from liver tissue. The crucial role of serum PAF-AH activity in the regulation of serum PAF levels has also been evaluated in patients with hyperbilirubinemic hepatobiliary disease. In this clinical study, apart from an elevated serum PAF-AH activity in all groups, a significant correlation with serum cholesterol and low density lipoprotein (LDL) cholesterol levels was also noted in patients with liver cirrhosis and/or obstructive cholestasis. These findings confirmed the hypothesis that serum cholesterol, especially the LDL fraction, is a major factor influencing serum PAF-AH activity in vivo. Although both PAF-AH activity/cholesterol and PAF-AH activity/LDL cholesterol ratios were elevated in all types of hyperbilirubinemic liver disease, the differential alteration of these ratios following normalization of bilirubin levels in each study group indicated that factors other than serum LDL levels, such as reduced biliary excretion of PAF-AH, might also be involved in the enhancement of PAF-AH activity.

Significantly higher blood PAF levels were observed in rats with experimental cirrhosis in a study evaluating the hemodynamic effects of PAF in cirrhosis. Cirrhotic animals demonstrated a hyperdynamic circulatory status, similar to that induced by systemic intravenous PAF infusion, with increased cardiac output (CO) and decreased mean arterial pressure (MAP) and peripheral vascular resistance (PVR). In these animals, intravenous administration of BN-52021 resulted in a significant decrease in CO, attributed to the preexisting cardiac failure becoming apparent, along with a significant increase in PVR to near normal values, whereas MAP was only slightly elevated. These results strongly suggested a possible role for PAF in the regulation of hemodynamic changes in liver cirrhosis. The response of hepatic macrophages and the effects of PAF in experimental thioacetamide (TAA)-induced cirrhosis have been studied by Noda et al. TAA administration resulted in an increase in hepatic macrophage population and cell size, followed by a marked increase in liver tissue hydroxyproline content, an index of collagen formation and deposition, indicative of cirrhosis. Intravenous PAF infusion during fibrosis induction before the development of cirrhotic changes significantly increased hepatic portal pressure and decreased oxygen consumption in TAA-treated animals compared to controls, possibly through elevation of the portal venous resistance by PAF-related molecules. The remarkable increase in prostaglandin D2 (PGD2) and thromboxane B2 (TXB2) levels in the perfusate induced by PAF was attributed to enhanced eicosanoid production and release by the increased population of active hepatic macrophages, suggesting a possible complex interaction between these components of chronic liver injury. Moreover, PAF-induced glycogenolysis was significantly lower in the TAA-treated group, indicating the disturbance of hepatocellular function in the early stages of cirrhosis as the likely cause of suppressed glucose production. Two serial studies on experimental carbon tetrachloride (CCl4)-induced cirrhosis have further elucidated the role of PAF and its cognate receptor in the pathophysiology of liver cirrhosis. The first study revealed a pronounced increase in both basal and ET-1-stimulated PAF synthesis in Kupffer cells from cirrhotic rats compared to controls. PAF binding capacity of these cells was also doubled, owing to increased density of functional PAF-Rs, although no significant difference in the PAF-R affinity was noted. It was also shown that Kupffer cells substantially contributed to increased hepatic and circulating PAF levels in cirrhosis. Consistent with the receptor binding data, the mRNA expression of PAF-R was also significantly increased. Since previous studies have shown that PAF stimulated eicosanoid synthesis in Kupffer cells, the enhanced production of prostaglandin E2 (PGE2), a hepatoprotective eicosanoid, observed in this study suggested an autocrine loop of PAF action which limited liver injury. The second study confirmed and extended these results in the hemodynamics of cirrhosis. Compared to control rats, cirrhotic ones presented higher hepatic and arterial blood PAF levels, elevated PAF-R mRNA transcription and protein expression levels and higher baseline portal pressure. PAF infusion caused an augmented increase in portal pressure, whereas administration of BN-52021 resulted in a significant decrease of portal pressure in cirrhotic animals, without influencing systemic arterial hypotension. In addition, increased PAF-R density was observed in the contractile perisinusoidal stellate cells from cirrhotic livers. These results suggested that elevated circulating PAF levels, derived mainly from liver tissue, largely contribute to portal hypertension which is further exacerbated by upregulation of PAF-Rs in the cirrhotic liver.

CONCLUSIONS

During the past two decades, the role of PAF in a number of physiological and pathophysiological processes has been extensively investigated and documented. The characterization of PAF-Rs and their attendant signaling pathways, involving a complex interactive network of cytokines, second messengers and autacoids, has further elucidated the extracellular and intracellular actions of this potent proinflammatory lipid mediator. Liver pathology appears to be directly related to PAF synthesis and release by local or systemic cellular sources. The implication of PAF in virtually all types of liver injury and regeneration has been well-established by numerous experimental and clinical studies. It has been explicitly shown that PAF is a major contributor to the initiation and prolongation of liver IR injury, liver graft dysfunction and various types of acute liver injury, while its involvement in cases of chronic liver injury is possibly more complicated due to interference of several other mediators. However, there remain many incompletely characterized or probably unknown aspects in regard to the crucial role of PAF in the regulation and modification of cellular responses to various endogenous and exogenous biological or biochemical stimuli. The effects of potent and specific PAF-R antagonists in experimental conditions associated
with liver injury have provided emerging evidence for their potentially beneficial use in the clinical setting. The need to establish standardized experimental, or even clinical, models for the evaluation of the role of PAF and its antagonism is imperative in order to limit the variability of current models and provide an accurate means for reliable comparisons. Moreover, the present review could ideally serve as a comprehensive basis for future reference in this intriguing field of research.

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