Role of Thromboxane in Producing Hepatic Injury During Hepatic Stress

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Hepatic injury after hepatic stress is caused by several mechanisms, including inflammatory reaction and microcirculatory disturbance. Levels of thromboxane, a vasoconstrictive eicosanoid, have been shown to increase in systemic circulation after different types of hepatic stress such as endotoxemia, hepatic ischemia-reperfusion, hepatectomy, liver transplantation, hemorrhagic shock and resuscitation, hepatic cirrhosis, and alcoholic liver injury. The production of thromboxane from the liver is also enhanced under these stresses, which may act on the liver in an autocrine or a paracrine fashion. Kupffer cells, resident hepatic macrophages, may be a major source of stress-induced thromboxane, although other cell types in the liver such as sinusoidal endothelial cells and hepatocytes may also produce this eicosanoid. Thromboxane induces hepatic damage through vasoconstriction, platelet aggregation, induction of leukocyte adhesion, up-regulation of proinflammatory cytokines, and induction of other vasoconstrictor release. In this regard, administration of cyclooxygenase inhibitor, specific thromboxane synthase inhibitor, and specific thromboxane receptor antagonists has been shown to protect from severe hepatic injury elicited by these hepatic stresses. Furthermore, blockade of Kupffer cell function by administration of gadolinium chloride showed salutary effects in preventing hepatic damage in bile duct ligation models. This review article summarizes the recent knowledge of the role of thromboxane in various types of hepatic stress and the effects of thromboxane inhibitors in these models.

Arch Surg. 2005;140:801-807

Hepatic damage induced by hepatic stress is mediated by various mediators, including vasoactive agents, proinflammatory cytokines, reactive oxygen species, and eicosanoids. The action of these mediators results in microcirculatory dysfunction, leukocyte infiltration, damage of cellular membranes, development of fibrosis, and stasis of biliary flow. Hepatic microcirculation is maintained by a fine balance between vasodilators and vasoconstrictors (Figure 1). Endothelin, thromboxane A2 (TXA2), angiotensin II, and catecholamine have been proposed as vasoconstrictors in the hepatic microcirculation, whereas nitric oxide (NO), carbon monoxide, and prostacyclin (prostaglandin I2 [PGI2]) have been proposed as vasodilators that counteract the effect of vasoconstrictors. Increased levels of circulating vasoconstrictors or decreased production of vasodilators results in a constriction of hepatic arterial and/or portal venous flow that leads to heterogeneous perfusion in the hepatic microcirculation. Unbalanced sinusoidal perfusion in the liver finally leads to an insufficient blood supply to the hepatic parenchyma, which leads to hepatocellular injury.

Eicosanoids are synthesized from arachidonic acid metabolism through the action of cyclooxygenase (COX) or lipoxigenase (Figure 2). The functioning groups of eicosanoids include prostaglandins, PG1, TXA2, leukotrienes, and lipoxins. In general, eico-
Eicosanoids act in an autocrine or a paracrine manner due to their unstable nature and very short half-life (<3 minutes). Among the eicosanoids, TXA2 has been shown to promote inflammatory process in the liver, vascular constriction in the portal venous system, and leukocyte adhesion in the sinusoids, all of which lead to hepatic tissue injury. Therefore, it could be postulated that TXA2 plays a key role in producing hepatic injury after various types of hepatic stress. The half-life of TXA2 is very short (approximately 30 seconds), as in other eicosanoids. Therefore, when TXA2 is produced by the liver, it should act in the liver in an autocrine or a paracrine fashion. Kupffer cells, resident macrophages in the liver, play a major role in the pathophysiology of hepatic damage under various kinds of hepatic stress. One of the roles of Kupffer cells in hepatic injury is production of TXA2 in response to stress (Figure 3).

Synthesis of TXA2 is regulated by COX, which catalyzes the conversion of arachidonic acid to prostaglandins and TXA2. Two isoforms of COX have been identified, COX-1, which is constitutively expressed in most of the tissues, and COX-2, which is inducible by a variety of stresses. In the liver, the expression of COX-2 has been shown to be induced by endotoxemia, ischemia-reperfusion, bile duct ligation, and alcoholic liver injury. The increased expression of COX-2 also produces increased production of TXA2 from the liver, which produces additional hepatic injury.

Because a specific TXA2 synthase inhibitor or TXA2 receptor (thromboxane-prostanoid [TP] receptor) antagonist blocks the action of TXA2, and because these drugs are widely used in the clinical arena without major adverse effects, it is important to determine whether blockade of TXA2 action leads to the attenuation of hepatic injury during hepatic stress. Furthermore, it appears essential to elucidate the precise mechanisms of TXA2-mediated hepatic injury after hepatic stress. In this article, we review and summarize recent knowledge on the role of TXA2 in producing hepatic injury under various hepatic stresses. The effects of TXA2 inhibitors on hepatic damage and an interaction between TXA2 and other vasoactive agents, which might further influence the effects of TXA2, are also discussed.

Endotoxemia produces severe hepatic damage through the activation of an inflammatory process and derangement of microcirculation. In this regard, TXA2 produced from the liver has been proposed as one of the responsible mediators. Administration of lipopolysaccharide (LPS) in the isolated perfused liver results in a rapid release of TXB2, a stable metabolite of TXA2, into the perfusate, suggesting that the liver is an important source of TXA2 after endotoxemia. In the liver, Kupffer cells are responsible for the increased production of TXA2, although sinusoidal endothelial cells could also serve as a source of TXA2 release in response to LPS administration. Other resident macrophages such as peritoneal macrophages and alveolar macrophages are also found to release TXA2 during endotoxemia. Thromboxane A2 is involved in endotoxemia-induced hepatic microcirculatory disturbance through its vasoconstrictive effect. Analogues of TXA2 not only directly contract the vascular smooth muscle cells but also contract the hepatic stellate cells, which have been shown to regulate sinusoidal perfusion. This action leads to a heterogeneous sinusoidal flow and impairs hepatic microcirculation. Enhanced release of TXA2 from the liver also has been shown to be responsible for the production of tumor necrosis factor α (TNF-α), a representative proinflammatory cytokine, from the liver. Furthermore, TXA2 is associated with the LPS-induced increase in intercellular adhesion molecule 1 expression in the intrahepatic vasculatures, which facilitates leukocyte adhesion and contributes to the enhancement of hepatic inflammatory process. These results indicate that the increased production of TXA2 from the liver and its autocrine or paracrine action on the liver contribute to hepatic injury in endotoxemia.
Thromboxane A2 plays a pivotal role in hepatic injury after ischemia-reperfusion. Numerous animal studies have shown that COX-1 inhibitors, COX-2 inhibitors, selective TXA2 synthase inhibitors, and specific TXA2 receptor antagonists protect the liver from injury after ischemia-reperfusion. These treatments reduce the damage to sinusoidal lining cells, ameliorate histological liver necrosis, lower the levels of serum transaminase, and restore hepatic tissue blood flow. Administration of PGI2 analogues, which counteract the action of TXA2 in vasoconstrictive and platelet-aggregating effects, also showed hepatoprotective effects in the hepatic ischemia-reperfusion model.

**Hepatic Resection.** Circulating TXB2 levels are remarkably increased during and after hepatic resection, and inhibition of TXA2 synthesis substantially reduces hepatic damage after hepatectomy. These effects have been shown in animal and human hepatectomy cases. In a canine model, the plasma levels of TXB2 increased significantly within 24 hours after 84% hepatectomy. This was associated with insufficient blood flow in the portal vein and hepatic tissue. However, administration of TXA2 synthase inhibitor dramatically improved the 2-week survival rate (from 12.5% to 58.3%) after 84% hepatectomy, which suggests the potential value of TXA2 inhibitor in extending the resectability of the liver. In human hepatectomy cases under hemihepatic vascular control, the levels of total prostanoids (6-keto-prostaglandin-F1α, prostaglandin E2, and TXB2) were significantly increased after hepatectomy, and these levels were positively correlated with the hepatic ischemic time. In the same study, administration of TXA2 synthase inhibitor reduced the plasma TXB2 levels and concomitantly reduced serum glutamic oxaloacetic transaminase levels. Thromboxane A2 is produced in the organ upstream from the liver and may act on the liver during and after hepatectomy. A report indicated that the spleen could be the source of TXA2 hypersecretion during hepatectomy because the level of TXB2 in the splenic vein was found to be significantly higher than that in the mesenteric vein.

**Ischemia-Reperfusion–Induced Hepatic Injury**

Hepatic damage after ischemia-reperfusion is a serious problem in a number of clinical settings, including hepatic resection, liver transplantation, and hemorrhagic shock and resuscitation. The mechanisms of ischemia-reperfusion–induced hepatic injury include the activation of inflammatory response, formation of reactive oxygen species, and microcirculatory disturbance. A variety of humoral mediators are included in these mechanisms such as TNF-α, interleukin 1β, interleukin 6, endothelins, prostaglandins, and TXA2.

**Ischemia-Reperfusion Model.** Thromboxane A2 plays a pivotal role in hepatic injury after ischemia-reperfusion. Numerous animal studies have shown that COX-1 inhibitors, COX-2 inhibitors, selective TXA2 synthase inhibitors, and specific TXA2 receptor antagonists protect the liver from injury after ischemia-reperfusion. These treatments reduce the damage to sinusoidal lining cells, ameliorate histological liver necrosis, lower the levels of serum transaminase, and restore hepatic tissue blood flow. Administration of PGI2 analogues, which counteract the action of TXA2 in vasoconstrictive and platelet-aggregating effects, also showed hepatoprotective effects in the hepatic ischemia-reperfusion model.

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Further, splenic macrophages after extensive hepatectomy produced more TXA2, and a splenectomy in conjunction with an extensive hepatectomy prevented remnant hepatic dysfunction.

**Liver Transplantation.** Vasoactive agents liberated from the liver after initiation of reperfusion have been proposed as the responsible factors underlying the pathophysiology of transplantation-related liver failure. The levels of prostanoids in the hepatic vein immediately after liver grafting are markedly increased, and this is significantly associated with poor early graft function. Thus, pretreatment with TXA2 synthase inhibitor not only significantly reduced the serum levels of TXA2 but also provided a better graft function and survival rate after liver transplantation. Kupffer cells have been shown to play an important role in eicosanoids production and in the activation of the inflammatory process and microcirculatory disturbance of liver grafts from non–heart-beating donors, which are strongly associated with reperfusion injury after liver transplantation. In this regard, suppression of Kupffer cell function substantially reduces graft damage after liver transplantation.

**Hemorrhagic Shock and Resuscitation.** Hemorrhagic shock and resuscitation is a hepatic hypoxemia-reoxygenation model. Although the extent of hypoxia in this model is relatively mild compared with the model of total vascular occlusion, prolonged mild hypoxemia results in liver damage similar to that observed after severe ischemia for a shorter interval. Previous studies have shown a decreased clearance of indocyanine green, increased plasma alanine aminotransferase levels, and impaired functional and metabolic activities of liver grafts even after a short period of hypoxia-reoxygenation. These results suggest that the extent of liver injury is dependent on the severity and duration of ischemia-reperfusion and the degree of microcirculatory dysfunction that occurs after reperfusion.
paired hepatic perfusion after trauma and hemorrhage. An isolated liver perfusion study after hemorrhage and resuscitation has shown that the release of TXB₂ from the liver is significantly increased, and these TXB₂ levels were correlated with the extent of hepatic damage.

**Hepatic Cirrhosis**

In hepatic cirrhosis, increased resistance in the portal venous system is the primary factor in the pathophysiology of portal hypertension. Increased resistance in the portal venous system is induced by the destruction of normal hepatic structure as a result of fibrin deposition or by the impaired balance between the action of vasodilators and vasoconstrictors. Studies have shown that cirrhotic livers exhibit hyperresponse to vasoconstrictors such as catecholamine, endothelin, and leukotriene D₄. In addition to these vasoconstrictors, the production of TXA₂ is enhanced in the cirrhotic liver and has been shown to be responsible for an increased resistance in the portal venous system. This phenomenon was observed in 2 different cirrhotic models such as bile duct ligation and carbon tetrachloride intoxication. The expression of COX-2 increases in both models; however, the expression of COX-1 does not change after stress. Furthermore, Kupffer cells have been implicated as a major source of TXA₂ in both models. In this regard, the blockade of Kupffer cell functions by gadoxilium chloride results in the attenuation of TXB₂ release from the bile duct ligation–induced cirrhotic liver. Furthermore, this decreased production of TXB₂ leads to the attenuation of portal hypertension and hepatic injury. Indomethacin, a nonspecific COX inhibitor, and specific TXA₂ receptor antagonists also showed the same protective effects on the liver as the blockade of Kupffer cell functions under cirrhotic conditions.

**Alcoholic Liver Disease**

Severity of liver injury in experimental alcoholic liver disease has been shown to be positively correlated with plasma levels of alcohol. Subsequent studies in the same laboratory showed increased expression of hepatic COX-2 in ethanol-fed animals. This enhanced COX-2 expression in the liver is associated with increased TNF-α messenger RNA expression, lipid peroxidation, and synthesis of TXB₂ in the liver. In this regard, treatment with TXA₂ receptor antagonists or TXA₂ synthase inhibitors decreased TNF-α production and prevented inflammatory changes in ethanol-fed rats. In experimental alcoholic liver disease, Kupffer cells are most likely the source of TXB₂, although endothelial cells and hepatocytes might also produce this prostanoid. Furthermore, the expression of TXA₂-receptor messenger RNA is highest in Kupffer cells compared with other cell types in the liver, suggesting the existence of autocrine effects of TXA₂ on Kupffer cells.

**EFFECT OF THROMBOXANES ON OTHER VASOACTIVE AGENTS**

A relationship between TXA₂ release and other vasoactive mediators has been described in a number of studies. Those mediators include endothelin and angiotensin II as vasoconstrictors and NO and PGI₂ as vasodilators. Endothelin, one of the most potent vasoconstrictors, has been shown to increase in circulation under various hepatic stresses. Increased production of endothelin activates vascular smooth muscle cells and hepatic stellate cells in the liver, which causes stenosis on the presinusoidal portal vein and sinusoids, respectively. Several studies have shown that the release of endothelin is regulated by TXA₂. Treatment of rat heart smooth muscle cells by a stable TXA₂ mimetic induces up-regulation of endothelin 1 messenger RNA. Increased serum endothelin levels after allograft liver transplantation are significantly decreased by the treatment with TXA₂ synthase inhibitors given at the time of liver harvesting. These results indicate that TXA₂ induces the release of endothelin, which may cause more potent and long-lasting vasoconstrictive effects. However, in other reports that used cultured endothelial cells, TXA₂ showed no effect on endothelin 1 secretion, whereas vasodilator prostaglandins such as prostaglandin E₂ and PGI₂ significantly inhibited endothelin 1 secretion. In view of these results, it can be postulated that these mechanisms may be tissue or cell type specific. The release of TXA₂ is also regulated by endothelin. Endothelin 1 evoked the release of endogenous TXA₂ from the cultured rabbit airway smooth muscle cells. Sustained contraction of rat aorta induced by endothelin at least partly requires persistent activation of TXA₂ receptors (TP receptors). In the liver, preadministration of indomethacin or a specific TXA₂ synthetase inhibitor significantly inhibits the decreases in hepatic blood flow induced by endothelin. Despite sufficient evidence of the interaction between endothelin and TXA₂, detailed mechanisms including the signaling pathway are still unknown, although protein kinase C may play some role in this interaction.

**Angiotensin II**

As with endothelin, angiotensin II is an effective vasoconstrictor for the hepatic vasculature and may be at least partly responsible for stress-induced hepatic injury. Angiotensin II receptors of the AT₁ subtype are present on the hepatic stellate cell, and the activation of the receptors induces stellate cell contraction. It also induces a proliferation and activation of hepatic stellate cells and promotes the process of liver fibrosis. Furthermore, chronic elevation of circulating angiotensin II levels damages the liver by activating proinflammatory events. In this regard, blockade of angiotensin II receptor leads to an attenuation of hepatic fibrosis and a reduction in inflammatory response–mediated hepatic injury under stressful conditions. Vasoconstriction induced by TXA₂ is significantly inhibited by an angiotensin receptor antagonist in the isolated human internal mammary artery, suggesting cross-interaction between the TP receptor and angiotensin II receptor. A TXA₂ synthase inhibitor causes a decrease in the vasoconstrictor effect of angiotensin II in the isolated rabbit kidney and abolishes edema produced by angiotensin II in the isolated rat lung. Although an interaction between TXA₂ and angiotensin II in the liver has
Nitric Oxide

In addition to vasoconstrictors, TXA2 also causes “cross talk” with the vasodilators.\(^{98}\) Up-regulation of inducible NO synthase (iNOS) and release of its product NO by Kupffer cells in the liver is markedly enhanced by LPS administration.\(^{99}\) Although the production of TXB2,\(^{100}\) is also induced by LPS, this effect is partly attenuated by NO.\(^{100}\) Therefore, an inhibition of NO synthesis results in a further increase in TXB2 production by Kupffer cells. The levels of TXB2 derived from platelets are also significantly increased in iNOS-deficient animals.\(^{101}\) These results indicated that NO has an inhibitory effect on TXA2 production. Other evidence showed the regulatory role of TXA2 on NO production. Vascular smooth muscle cells, when stimulated with LPS or proinflammatory cytokines, showed exaggerated NO production due to up-regulation of iNOS. However, endogenous TXA2 reduced iNOS expression and NO production.\(^{102}\) Cytokine-induced NO production was significantly higher in TP receptor knockout mice. Furthermore, vasodilating prostanoids such as prostaglandin E2 and PGI2 have been shown to increase iNOS expression and thus antagonize TXA2 action.\(^{103}\) These results indicate the existence of a mutual inhibitory mechanism between NO and TXA2. However, this mechanism is not available in cirrhotic livers. Although COX-derived TXA2 plays a major role in methoxamine-induced portal venous hyperresponse in cirrhotic livers, this is not modulated by an inhibition of NO production.\(^{71}\) An increased production of TXA2 from the liver is involved in the pathogenesis of endothelial dysfunction in cirrhotic rat livers.\(^{72}\) However, this is not affected by NO synthase inhibitor, indicating a lack of regulatory mechanism between NO and TXA2 in cirrhotic livers.

Prostacyclin

Prostaglandin I\(_2\) induces vasodilation, inhibits platelet aggregation, and antagonizes the effects of TXA2. The plasma TXA2/PGI2 ratio changes in some pathological conditions.\(^{104}\) It increases during liver ischemia and could influence the extent of hepatic injury\(^{105}\) and survival rate of animals.\(^{106}\) Therefore, administration of PGI2, which reduces the TXA2/PGI2 ratio, improves the outcome of ischemia-reperfusion–induced hepatic injury.\(^{54}\) Administration of PGI2 after liver transplantation also improved hepatic-splanchnic oxygenation and reduced reperfusion injury in human randomized controlled trials.\(^{107}\) Moreover, modulation of the TXA2/PGI2 ratio by inhibiting TXA2 production or by administering PGI2 analogue significantly ameliorated the functional impairment of the residual liver after major hepatectomy.\(^{59}\) The specific TP antagonists have been shown to protect hepatic tissue from ischemia-reperfusion injury by increasing PGI2 levels.\(^{50}\) A recent study\(^{108,109}\) showed that the activation of PGI2 receptors in platelets completely inhibits TXA2-mediated TP receptor signaling in a protein kinase A–dependent but not a protein kinase C–dependent manner, suggesting inhibitory effects of PGI2 on TXA2 action. This is mediated through the activation of TP\(_{\alpha}\) but not TP\(_{\beta}\) receptors in platelets.\(^{109}\)

CONCLUSIONS

The mechanisms of hepatic injury during hepatic stress have been extensively investigated. The available information from clinical and animal studies strongly indicates that TXA2 plays a pivotal role in producing hepatic injury during various forms of stress. Moreover, TXA2 up-regulates other vasoconstrictors and down-regulates vasodilators, and these effects further exacerbate the vasoconstrictive effects of TXA2 (Figure 4). Nonetheless, blockade of TXA2 action prevents the hepatic damage elicited by these stresses. It is, however, disappointing to note that only a few randomized clinical trials have been performed to determine the usefulness of thromboxane inhibitors in preventing hepatic injury. Such trials should be undertaken in the future because thromboxane inhibitors are relatively safe and are not expected to produce any deleterious effects in the patients. Moreover, additional studies to elucidate the precise mechanism of TXA2-mediated hepatic injury should be performed to establish more effective therapy against hepatic injury after various adverse circulatory conditions.

Accepted for Publication: October 8, 2004.

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Funding/Support: This study was supported by grant R37 GM 39519 from the National Institutes of Health, Bethesda, Md.

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