Role of thromboxane A2 in early BDL-induced portal hypertension

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Yokoyama, Yukihiro, Hongzhi Xu, Nicole Kresge, Steve Keller, Amir H. Sarmadi, Rajiv Baveja, Mark G. Clemens, and Jian X. Zhang. Role of thromboxane A2 in early BDL-induced portal hypertension. Am J Physiol Gastrointest Liver Physiol 284: G453–G460, 2003. First published November 13, 2002; 10.1152/ajpgi.00315.2002.—Although the mechanisms of cirrhosis-induced portal hypertension have been studied extensively, the role of thromboxane A2 (TXA2) in the development of portal hypertension has never been explicitly explored. In the present study, we sought to determine the role of TXA2 in bile duct ligation (BDL)-induced portal hypertension in Sprague-Dawley rats. After 1 wk of BDL or sham operation, the liver was isolated and perfused with Krebs-Henseleit bicarbonate buffer at a constant flow rate. After 30 min of nonrecirculating perfusion, the buffer was recirculated in a total volume of 100 ml. The perfusate was sampled for the enzyme immunoassay of thromboxane B2 (TXB2), the stable metabolite of TXA2. Although recirculation of the buffer caused no significant change in sham-operated rats, it resulted in a marked increase in portal pressure in BDL rats. The increase in portal pressure was found concomitantly with a significant increase of TXB2 in the perfusate (sham vs. BDL after 30 min of recirculating perfusion: 1,420 ± 803 vs. 10,210 ± 2,950 pg/ml; P < 0.05). Perfusion with a buffer containing indomethacin or gadolinium chloride for inhibition of cyclooxygenase (COX) or Kupffer cells, respectively, substantially blocked the recirculation-induced increases in both portal pressure and TXB2 release in BDL group. Hepatic detection of COX gene expression by RT-PCR revealed that COX-2 but not COX-1 was upregulated following BDL, and this upregulation was confirmed at the protein level by Western blot analysis. In conclusion, these results clearly demonstrate that increased hepatic TXA2 release into the portal circulation contributes to the increased portal resistance in BDL-induced liver injury, suggesting a role of TXA2 in liver fibrosis-induced portal hypertension. Furthermore, the Kupffer cell is likely the source of increased TXA2, which is associated with upregulation of the COX-2 enzyme.

hepatic fibrosis; vasoconstriction; isolated liver perfusion; cyclooxygenase; portal pressure

CIRRHOSIS IS A COMMON SEQUELA of severe chronic liver diseases including chronic hepatitis, obstructive biliary diseases, and alcoholic cirrhosis. It is well documented that a hepatic upregulation of the constrictor endothelin and a decreased release of the vasodilator nitric oxide (NO) increase portal venous resistance, resulting in portal hypertension in cirrhosis (21, 23, 35, 37, 38). However, the possible role of thromboxane A2 (TXA2), another potent constrictor from cyclooxygenase (COX)-dependent arachidonic acid metabolism, in the development of portal hypertension in cirrhotic liver has not been explicitly examined.

TXA2 has been reported to function as a potent vasoconstrictor in the portal circulation system (9, 42). Various studies have shown increased production of the COX products from chronically injured livers in both animal and human models (7, 10, 11, 22, 26, 28, 44, 46, 47). Increased levels of TXA2 have been reported in the systemic circulation after bile duct ligation (BDL)-induced cirrhosis (15). Upregulation of TXA2 in bile duct-obstructed cirrhosis was also observed in the kidney and lung, which contributed to impaired renal (6) and pulmonary function (19).

Endothelial cells and Kupffer cells are the primary sources of COX-dependent eicosanoids in the diseased liver (8, 29). The elevated production of eicosanoids is normally associated with upregulation of key enzymes such as COX in the biosynthesis pathway. A recent study by Alric et al. (1) showed a significant release of thromboxane B2 (TXB2), a stable metabolite of TXA2, from isolated Kupffer cells of the CCl4-induced cirrhotic rat liver. Another study by Nanji et al. (25) found increased mRNA levels of COX-2, the inducible form of the COX enzyme, in rat liver samples of CCl4-induced cirrhosis, suggesting an upregulation of COX-2 enzyme. Although evidence from these previous studies has suggested the increased production of TXA2 in the cirrhotic liver and its possible role in the pathogenesis of cirrhosis, the exact involvement of TXA2 in the development of portal hypertension in cirrhosis is unclear. Therefore, the purpose of the present study was twofold. First, we sought to determine whether the COX-dependent production of TXA2 in the liver increases in an early stage of liver fibrosis-induced portal hypertension by using a BDL-induced liver injury model in rats. Second, we tested whether Kupffer cells were the major cell type responsible for the production...
of TXA₂ in BDL-induced portal hypertension by eliminating Kupffer cells with an inhibitor, gadolinium chloride (GdCl₃).

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats weighing 250–300 g were housed in a temperature-controlled animal facility with alternating 12:12-h light-dark cycles and fed standard lab chow ad libitum with free access to water. All procedures were performed in accordance with National Institutes of Health guidelines under a protocol approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Charlotte.

**BDL-induced portal hypertensive rat liver model.** For BDL, the animals were anesthetized by halothane inhalation. After a midline laparotomy, the common bile duct was doubly ligated with 4-0 silk and was transected between the two ligations. Sham operation was performed similarly, with the exception of ligating and transecting the bile duct. The abdomen was closed in two layers using 4-0 silk, and animals were allowed to have free access to food and water during recovery. All of the following experiments were performed after 1 wk of surgery. One week of BDL was chosen for the experiment because preliminary studies showed that 1) the portal hypertension was well developed at this time point; 2) the injury within the liver was mild but evident; and 3) no overt structural changes in the portal circulation system were observed, although an inflammatory response around the portal triads was obvious.

**Histology studies.** After 1 wk of BDL or sham operations, whole liver tissue samples were harvested for histological analysis. The pieces of liver tissue were immersed immediately in 10% buffered formalin overnight and thereafter washed with 70% ethanol three times. Subsequently, the tissue was dehydrated in a graded series of ethanol and embedded in paraffin. Six-micrometer-thick sections were mounted on glass slides and stained with hematoxylin and eosin for light microscopy analysis.

**In vivo portal pressure measurement and alanine aminotransferase and lactate dehydrogenase assays.** After 1 wk of sham or BDL surgery, the rats were fasted overnight but allowed free access to water. The rats were anesthetized with pentobarbital sodium (50 mg/kg body wt ip), and the portal pressure was measured by inserting a PE-50 catheter from the splenic vein into the portal vein. Subsequently, blood samples were obtained from the abdominal aorta of BDL and sham rats for determination of serum alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). After the plasma was separated by centrifugation for 2 min at 5,000 rpm, measurements were made spectrophotometrically using diagnostic kits from Sigma (St. Louis, MO).

**Isolated liver perfusion.** In separate experiments, the rats were subjected to isolated liver perfusion. Sham and BDL groups were assigned into 1) a nontreatment group (control: sham, n = 11; BDL, n = 14); 2) an indomethacin perfusion group (Indo: sham n = 7; BDL, n = 7); 3) a furegrelate pretreatment group (sham, n = 4; BDL, n = 6); and 4) a GdCl₃ pretreatment group (sham, n = 5; BDL, n = 8). The liver was exposed through a wide transverse incision, and the portal vein was isolated. After the portal vein was cannulated with a PE-240 catheter, the liver was perfused with Krebs-Henseleit bicarbonate buffer (in mM: 118 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 25 NaHCO₃, 0.1 EDTA, and 2.5 CaCl₂, pH 7.4) for 10 min to wash out the blood. In the Indo group, 50 μM indomethacin was added in the perfusate to inhibit the production of cicosanoids. In separate experiments, the specific TXA₂ synthase inhibitor furegrelate [5-(3-pyridinylmethyl)bensofurancarboxylic acid; 1 mg/100 g body wt] was injected intraperitoneally 14 h before the experiment, and a boosting dose (5 μM) was added in the perfusate during perfusion to inhibit TXA₂ production. In the GdCl₃ group, 10 mg·ml⁻¹·kg body wt⁻¹ GdCl₃ dissolved in saline was injected through the penile vein 24 h before the perfusion to inhibit the function of Kupffer cells. The isolated liver perfusion was performed using a constant flow rate (100 ml·min⁻¹·kg body wt⁻¹), as described previously with minor modification (2). Briefly, warmed perfusate was pumped from a reservoir into an overflow chamber and oxygenated via a silastic tubing oxygenator (95% O₂ + 5% CO₂). The temperature of perfusate was maintained at 36–37°C by warming the reservoir in a water bath. A pressure transducer was placed in line immediately before the portal inlet cannula to monitor portal pressure. The liver was perfused for 30 min with a nonrecirculating system followed by 80 min of recirculating perfusion. The total volume of perfusate during recirculating perfusion was 100 ml. Perfusion was sampled before the start of recirculation (0-min point) and after 30 min of recirculation for the measurement of TXB₂ levels and LDH activity.

**Enzyme immunoassay for TXB₂.** Enzyme immunoassay kits (Assay Designs, Ann Arbor, MI) were used to determine the concentration of TXB₂ (stable TXA₂ metabolite) in the perfusate. The samples were diluted 1:10 with Krebs buffer so that the highest concentration of TXB₂ in the perfusate fell in the linear range of the standard curve. The level of TXB₂ was expressed as picograms per milliliter.

**RNA preparation and semiquantitative RT-PCR.** Total RNA was isolated from ~100 mg of liver tissue using RNA-STAT (TEL-TEST, Friendswood, TX), treated with 10 U/μl of RNase-free DNase (Boehringer Mannheim, Indianapolis, IN) in 10 mM MgCl₂, 20 mM Tris-HCl at 37°C for 30 min, and purification of RNA with RNA-STAT was repeated. Final RNA was dissolved in deionized water and stored at −80°C. Before the RT-PCR step, RNA samples from experimental groups were standardized by the intensity of ribosomal RNA bands in the RNA aliquots stained with ethidium bromide and electrophoresed in 1.5% agarose gel.

Reverse transcription of 1 μg of RNA to cDNA was carried out using GenAmp’s RNA PCR core kit (Perkin-Elmer, Branchburg, NJ) in a 20-μl volume according to the protocol from the manufacturer. Preliminary data showed that the concentration of 1 μg RNA for the genes studied was in the linear range of amplification. The final cDNA samples were diluted 1:2 with deionized RNAase-free water before PCR. The oligonucleotides made by GIBCO-BRL Life Technologies (Gaithersburg, MD) were used for PCR.

In the PCR reaction, 10 μl of diluted cDNA sample were amplified with gene-specific primers in a total volume of 25 μl. The following oligonucleotides were used as primers in the amplification reaction: GAPDH protein, sense 5'-TCCCT-CAAGATTGTGACCAA and antisense 5'-AGATCCCAACAAG-GATACATT; COX-1, sense 5'-CTGCTATGTTGCTATGCT- CATC and antisense 5'-AGGACCCGTCATCTCCGGGT-AATC; COX-2, sense 5'-CAAGACGTGCAAAGCCTCATT and antisense 5'-TAGTCTGGTAGGAGCCTTGCG.

The final reaction concentrations were: primers, 1 μM; KCl, 50 mM; Tris·HCl, 10 mM (pH 9.0); MgCl₂, 1.5 mM; dNTPs, 300 μM each; Taq DNA polymerase, 0.5 U per reaction. PCR was performed with 26 cycles for GAPDH (94°C, 45 s; 65°C, 45 s; 73°C, 1 min) and with 33 cycles for COX-1 and 38 cycles for COX-2 (94°C, 30 s; 54°C, 30 s; 73°C, 1 min), with initial incubation at 95°C for 3 min and final extension at 72°C for 5 min. To ensure equal loading from control and
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Fig. 1. Histological changes in sham-operated (A) and bile duct-ligated (BDL; B) livers following 1 wk of operation. The micrographs are hematoxylin and eosin staining of paraffin-embedded liver slides (×20). PT, portal triad; CV, central venule.

Histological changes. Although fibrosis was not prominent, extensive proliferation of bile duct and inflammatory cell infiltration around the portal triads were observed in the BDL group (Fig. 1). Micronodular fibrosis and reduction in sinusoidal density, which are typical after a prolonged period of BDL, were absent at 7 days after BDL. No other significant changes in sinusoidal structures were evident in the BDL group compared with the sham group.

Liver injury. After 1 wk of BDL, plasma ALT and LDH levels showed significant increases compared with sham (sham vs. BDL: ALT, 16.6 ± 2.1 vs. 99.4 ± 12.3 U/l; LDH, 108.2 ± 26.9 vs. 216.9 ± 23.4 U/l; *P < 0.05, n = 6 in each group), suggesting that mild but significant hepatocellular injury was present at this time point in the BDL liver (Fig. 2).

Portal hemodynamics. There was no significant difference in mean arterial pressure between sham and BDL in vivo. However, portal venous pressure was significantly higher in BDL than in sham (n = 6 in each group), suggesting that portal hypertension had already been established following 1 wk of BDL despite
the fact that morphological changes in the hepatic vasculature were not obvious. Markedly higher portal pressure in BDL was also observed in the isolated perfused liver (Fig. 3, A and B). During the 30 min of the nonrecirculation period, the portal pressure did not show significant changes in either the sham or the BDL group (Fig. 3, A and B). The portal pressure in the sham-operated animals during nonrecirculation showed no differences from the baseline in control, Indo, furegrelate, and GdCl3 groups (Fig. 3A). However, in the BDL animals, significantly lower portal pressure was observed at 0 min in Indo, furegrelate, and GdCl3 groups (Fig. 3A). After the beginning of recirculation, the portal pressure in the nontreated BDL group started to increase and showed significant increases at 10, 20, and 30 min compared with that at 0 min (Fig. 3B). The portal pressure reached its peak around 20 min before it started to decline. At 80 min of recirculation, the pressure returned to almost the same level as at 0 min. Interestingly, the portal pressure during the nonrecirculation was significantly lower in Indo, furegrelate, and GdCl3 groups than that in the nontreated BDL group. The increase in portal pressure seen during the recirculating perfusion in the nontreated BDL group was almost completely abolished in Indo, furegrelate, and GdCl3 groups in BDL rats (Fig. 3B). The pressure change during the recirculation was not observed in the sham group, which exhibited steady portal pressure throughout the entire perfusion (Fig. 3A).

LDH levels were measured in the perfusate to evaluate hepatic tissue damage (Fig. 4). LDH in the perfusate showed significantly higher levels in the nontreated BDL group compared with the nontreated sham group. High levels of LDH in the BDL group were significantly reduced in either the Indo-BDL or the GdCl3-BDL group, suggesting a contributing role of eicosanoids from Kupffer cells to hepatoportal injury.

**Perfusate TXB2 levels.** The data from the isolated liver perfusion experiment strongly indicated that vasoconstrictive substance(s) were released in BDL livers and the substance(s) might be COX-dependent eicosanoids. Therefore, we determined the release of TXA2 from the isolated perfused liver by measuring the levels of TXB2 in the perfusate using enzyme immunoassay. TXB2 levels at 0 min were significantly higher in the BDL group compared with the sham group (sham vs. BDL: 667 ± 501 vs. 3,191 ± 641 pg/ml; P < 0.05; Fig. 5A). The level of TXB2 after 30 min of recirculation was further increased in the BDL group, whereas the sham group did not show a significant change (sham vs. BDL: 536 ± 160 vs. 10,350 ± 1,591 pg/ml; P < 0.05; Fig. 5B). This enhanced production of TXB2 from the BDL liver was significantly attenuated in both the Indo-BDL and GdCl3-BDL groups, suggesting Kupffer cells as a major source of TXB2 production in the BDL liver (Indo-BDL and GdCl3-BDL: 194 ± 194 and 281 ± 88 pg/ml, respectively). The level of TXB2 at 80 min of recirculation in the nontreatment-BDL group did not show further elevation compared with the level at 30 min (data not shown).

**COX enzyme expression.** To determine whether the increased release of TXA2 was a result of upregulation of COX enzymes during the early stages of portal hypertension in cirrhosis, we measured the gene expressions of COX-1 and -2 using RT-PCR analysis. After 7 days of BDL treatment, the COX-1 mRNA level was significantly reduced in both the Indo-BDL and GdCl3-BDL groups, suggesting a contributing role of COX-2 production in the BDL liver (Indo-BDL and GdCl3-BDL: 194 ± 194 and 281 ± 88 pg/ml, respectively). The level of TXB2 was further increased in the nontreatment-BDL group compared with the level at 30 min (data not shown).

**LDH level in perfusate 30 min after the recirculation of sham-operated and BDL liver.** Sham, nontreatment + sham operation (n = 11); BDL, nontreatment + BDL (n = 14); Indo-BDL, indomethacin + BDL (n = 7); GdCl3-BDL, gadolinium chloride pretreatment + BDL (n = 8). *P < 0.05 vs. BDL.
increased in the BDL liver compared with sham-operated liver \( (P < 0.05) \), strongly suggesting that an upregulation of COX-2 but not COX-1 contributed to the enhanced TXA2 production in the BDL-induced early fibrosis.

The upregulation of COX-2 in BDL liver was further confirmed in protein levels by Western blot. COX-2 protein level was significantly increased following 7 days of BDL treatment compared with sham treatment (sham vs. BDL: 2.43 ± 0.75 and 6.21 ± 1.24 arbitrary units, respectively; \( P = 0.039 \); Fig. 7), suggesting that an upregulation of COX-2 protein levels contributed to the increased TXA2 production in the 7-day BDL model of cirrhosis.

DISCUSSION

Portal hypertension is commonly associated with cirrhosis. The mechanism by which portal hypertension develops in cirrhosis has been associated with overproduction of endothelins and underproduction of NO as a result of dysfunctional NO synthase in the cirrhotic liver (12, 16, 18, 27, 30, 34, 37). However, the role of TXA2 in the cirrhosis-induced portal hypertension has not been explicitly explored. Therefore, the present study was performed to elucidate the role of TXA2 in the development of portal hypertension. More specifically, the potential role of TXA2 was explored in an early stage of portal hypertension by using a BDL rat model. One week of BDL was chosen in this study for the reasons stated previously in MATERIALS AND METHODS. Most importantly, we sought to determine whether TXA2 is involved in the early functional changes in the prefibrotic liver that lead to portal hypertension before all of the structural abnormalities become significant.

Seven days after BDL, no overt structural changes in sinusoids were evident in the histological sections of the BDL liver. The only visible morphological changes were found around the portal triads with inflammatory cell infiltration and proliferation of bile ducts. The “normal” sinusoidal structures at 7 days after BDL were also observed using intravital fluorescence microscopy (data not shown). Despite the minimum morphological changes found in the 7-day BDL liver, portal pressure in those rats, detected both in vivo and in the isolated perfusion system, was significantly higher than in the sham-operated rats, confirming the development of portal hypertension (Fig. 3).

To explore the mechanism of the elevated portal pressure in the BDL liver, we employed the isolated
liver perfusion system with initial nonrecirculation followed by recirculation. Thirty minutes of nonrecirculating perfusion did not significantly change the portal pressure, whereas the portal pressure started to increase in the BDL liver as soon as the perfusion was switched to recirculation (Fig. 3). The elevation of the portal pressure during recirculation indicated that vasoconstrictive substances were released into the portal circulation and that they accumulated in a total volume of 100 ml Krebs buffer solution, resulting in an increase in the portal venous resistance. Indeed, when we measured the levels of TXB2 as a stable metabolite of TXA2 in the perfusate using enzyme immunoassay, it was found that this potent vasoconstrictor was markedly increased at 30 min of recirculation in the portal circulation of the BDL liver (Fig. 5). In contrast, no significant change of TXB2 levels was detected throughout recirculating perfusion in sham-operated livers.

TXA2, reportedly acting as a vasoconstrictor in the liver, is derived from arachidonic acid via a COX-dependent pathway. In the present study, we were able to inhibit the increase in the production of TXA2 from the BDL liver as evidenced by an almost complete blockade of TXB2 release at 30 min of recirculation using the COX inhibitor indomethacin. The complete blockade of TXA2 production with indomethacin was also reflected by the marked attenuation of the increase in portal pressure in the Indo-BDL group (Fig. 3). This suggests that the spontaneous release of TXA2 is largely responsible for the increased portal pressure during the recirculation. This was also confirmed by the results using the specific TXA2 synthase inhibitor furegrelate, which completely blocked the increase in portal pressure during the isolated perfusion in BDL livers. Even at baseline before recirculation, the release of TXA2 was significantly elevated (Fig. 3), which likely contributes to the portal hypertension observed in the BDL-induced early fibrotic liver injury, because indomethacin lowered the baseline portal pressure (Fig. 3). The present study examined specifically the role of TXA2 in portal hypertension of early fibrosis. It is unclear whether other COX-dependent eicosanoids are also involved in the pathogenesis of portal hypertension in BDL-induced cirrhosis.

It has been reported that prostacyclin is elevated in both the systemic and portal circulation in human cirrhosis and several animal models of portal hypertension (13, 26, 41, 45). It is believed that an increased production of the vasodilator prostacyclin is partly responsible for the hemodynamic changes in portal hypertension. The sources of the increased prostacyclin production are likely from the extrahepatic sites. In a recent study, Potenza et al. (31) found an upregulation of COX-1 and -2 in aorta and mesenteric vascular beds in portal hypertensive rats and suggested that generation of the endothelial vasodilator eicosanoids from these isoforms accounts for the splanchnic hyperemia in portal hypertension. However, whether significant amounts of hepatic prostacyclin are produced in cirrhosis-induced portal hypertension remains unclear. Our data using the TXA2 synthase inhibitor furegrelate argue against the possibility of significant hepatic production of vasodilator eicosanoids in BDL-induced portal hypertension, because no change was observed in the portal perfusion pressure during recirculation in the presence of furegrelate (Fig. 3). Nevertheless, further study is needed to clarify the role of prostacyclin in the pathogenesis of intrahepatic portal hypertension in cirrhosis and its interaction with TXA2.

The elevated production of TXA2 is associated with upregulation of key enzymes of the TXA2 biosynthesis pathway such as COX. COX is normally present in the liver in two isoforms: COX-1 and -2, the constitutive form and inducible form, respectively. The results of RT-PCR analysis showed that COX-2 but not COX-1 mRNA was upregulated in the liver of 7-day BDL rats. We, therefore, further determined whether the COX-2 enzyme is upregulated in the BDL-induced injury of the prefibrotic liver by performing Western blot analysis. Our results clearly showed that COX-2 was upregulated in the liver at 7 days following BDL (Fig. 7), strongly indicating that stimulation of COX-2 protein expression is at least in part responsible for the enhanced production of TXA2 in the BDL-induced injury of the prefibrotic liver.

Kupffer cells and endothelial cells have been reported as a major source of thromboxane in the liver (3, 4, 7, 8, 11, 14, 17, 29, 32, 33, 36, 43). Production of TXA2 from Kupffer cells is especially enhanced in pathological conditions such as endotoxemia, ethanol intoxication, and hypoxia (3, 14, 17, 43). A recent study by Alric et al. (1) showed a significant release of TXB2 from the isolated Kupffer cells of the CCl4-induced rat liver.

Fig. 7. Western blot analysis of COX-2 in sham-operated and BDL liver. One hundred micrograms of protein samples were separated by SDS-PAGE on a 12% acrylamide gel, and proteins were transferred to nitrocellulose membranes. The blots were probed with an anti-COX-2 monoclonal antibody and were exposed to the enhanced chemiluminescent treatment (A). The average densitometric quantification of Western blot signal intensities are shown in bar graphs (B). Data are means ± SE of 6 separate experiments in sham and BDL groups. *P < 0.05 vs. sham group.
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cirrhosis. However, to our knowledge, the effect of BDL on TXA\textsubscript{2} release from the Kupffer cells has never been reported. With the use of the Kupffer cell inhibitor GdCl\textsubscript{3} in the present study, we were able to completely eliminate the increased production of TXA\textsubscript{2} into the portal circulation (Fig. 5). Furthermore, the inhibition of TXA\textsubscript{2} production was accompanied by almost complete abolishment of the increased portal pressure by recirculation (Fig. 3) and decreased hepatocellular injury evidenced by the marked reduction of LDH (Fig. 4). It is worth noting that the reduction of liver injury by the inhibition of TXA\textsubscript{2} production with indomethacin or GdCl\textsubscript{3} might have helped prevent portal perfusion pressure from rising during the recirculating perfusion, which may constitute part of the hemodynamic effect of TXA\textsubscript{2} inhibition on the portal circulation.

These results strongly suggest a pathogenic role of the Kupffer cell in portal hypertension during the development of cirrhosis. The complete inhibition of TXA\textsubscript{2} production from Kupffer cells with GdCl\textsubscript{3} also argues against the possibility of the endothelial cells in the diseased liver being a significant source of TXA\textsubscript{2} production. However, one cannot exclude the possibility that factors released from the endothelial cells trigger the production of TXA\textsubscript{2} from Kupffer cells. For instance, endothelins and NO have been reported to regulate or modify the release of COX-dependent eicosanoids, including TXA\textsubscript{2}. Several groups reported that both ET-1 and -3 induce the release of eicosanoids such as PGE\textsubscript{2}, PGF\textsubscript{2}\alpha, and TXA\textsubscript{2}, which potentiate the vasoconstriction in the dog and rat renal arterioles (5, 24, 40). Kurihara et al. (20) showed that ET-3-stimulated decrease in hepatic blood flow was partially mediated by the release of TXA\textsubscript{2}. In contrast, NO was reported to inhibit production of PGE\textsubscript{2} and TXB\textsubscript{2} in LPS and IFN-\gamma-activated Kupffer cells (39). It is, therefore, apparent that the mechanisms underlying the production of TXA\textsubscript{2} in Kupffer cells remain elusive, and further study on the cross-talk between endothelial cells and Kupffer cells in the production of TXA\textsubscript{2} in the diseased liver may prove to be valuable.

Interestingly, our data on the changes in portal pressure on the recirculating perfusion in the BDL liver showed that the peak increase occurred between 20 and 30 min, which we attribute to the increased release of TXA\textsubscript{2}. However, after the peak increase, the portal pressure declined to almost the baseline level, although the same levels of TXA\textsubscript{2} remained in the recirculating perfusate. These results can be explained by several possibilities. The decline of portal pressure could result from the receptor downregulation. It is also possible that vasodilatory eicosanoids may be produced in a parallel or a delayed fashion. Another possibility is that other vasodilators, such as NO or carbon monoxide, were produced in response to the accumulation of TXA\textsubscript{2} in the portal circulation and thus counteracted the vasoconstrictive effect of TXA\textsubscript{2}. However, the interactions between prostanoids and NO or carbon monoxide are far from clear and remain to be elucidated.

In summary, the results of the present study provide novel information on the hepatic release of thromboxane in the portal circulation of the bile duct-obstructed liver, which suggests an important role of thromboxane in the pathogenesis of portal hypertension and the hepatic cellular injury in early fibrosis. Furthermore, the Kupffer cell may likely be the major source of thromboxane production in BDL-induced portal hypertension. Collectively, the data from the current study indicate that increased thromboxane release into the portal circulation is at least part of the instigating mechanism that leads to portal hypertension, and reduction of thromboxane production in the fibrotic liver may be therapeutically targeted for prevention and treatment of portal hypertension in fibrosis and cirrhosis.

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