Role of thromboxane in producing portal hypertension following trauma-hemorrhage

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Yokoyama, Yukihiro, Balazs Toth, William C. Kitchens, Martin G. Schwacha, Kirby I. Bland, and Irshad H. Chaudry. Role of thromboxane in producing portal hypertension following trauma-hemorrhage. Am J Physiol Gastrointest Liver Physiol 285: G1293–G1299, 2003; 10.1152/ajpgi.00268.2003.—Thromboxane A2 (TXA2) and endothelin-1 (ET-1) have been proposed as the important vasoconstrictors that increase portal venous resistance in paracrine or autocrine fashion. We hypothesized that the hepatic damage following trauma-hemorrhage (T-H) is induced by the impaired hepatic circulation due to the increased production of vasoconstrictors such as ET-1 and TXA2 by the liver. To test this, male Sprague-Dawley rats (n = 6/group) were subjected to trauma (i.e., midline laparotomy) and hemorrhage (35–40 mmHg for 90 min followed by fluid resuscitation) or sham operation. At 2 or 5 h after the end of resuscitation, the liver was isolated and perfused and portal inflow pressure, bile flow, and release of ET-1 and thromboxane B2 (TXB2; a stable metabolite of TXA2) into the perfusate were measured. The level of portal pressure was higher at 5 h following T-H compared with 2 h after T-H and sham. The portal pressure was inversely correlated to the amount of bile production. Furthermore, the bile flow was significantly correlated to the hepatic damage as evidenced by release of lactate dehydrogenase into the perfusate. The level of ET-1 at 5 h following T-H in the perfusate after 30 min of recirculation did not show any difference from sham. However, the levels of TXB2 in the T-H group were significantly higher than those in sham at that interval. These results indicate that the increased release of TXA2 but not ET-1 following T-H might be responsible for producing the increased portal resistance, decreased bile production, and hepatic damage.

endothelin; portal venous pressure; bile flow

THE FINE BALANCE BETWEEN VASOCONSTRICTORS and vasodilators maintains hepatic microcirculation. Increased circulating vasoconstrictors induced by a variety of stresses result in microcirculatory disturbance and subsequent hepatic damage. These stresses include endotoxia (11, 45), hepatic ischemia-reperfusion (14, 44), cirrhosis (23, 40), and hemorrhagic shock and resuscitation (31). Our previous studies (42) have indicated depressed hepatic function following trauma-hemorrhage, as evidenced by decreased clearance of indocyanine green (ICG) in rats, and also hepatic damage, as reflected by increased presence of plasma alanine aminotransferase (ALT) following trauma-hemorrhage. Moreover, disturbed hepatic perfusion was observed despite an increased recovery of cardiac output by volume resuscitation (43). These observations suggest that the increased hepatic vascular resistance may be one of the factors leading to hepatic damage following hemorrhagic shock and resuscitation. Endothelin-1 (ET-1) and thromboxane A2 (TXA2) have been reported to be potent vasoconstrictors in the intrahepatic circulation (5, 20, 41). These vasoconstrictors are produced in the liver in response to a variety of hepatic stresses and act on the hepatic circulations in an autocrine or paracrine fashion (9, 17, 27, 47). In this regard, it has been proposed that these vasoconstrictors impair the hepatic microcirculation and induce hepatic damage due to insufficient blood flow. Increased responsiveness of the portal circulation to ET-1, reflected by the increased portal resistance, was observed in response to the lipopolysaccharide injection in the rat model (29). A similar response was observed in the hepatic ischemia-reperfusion model (14). Studies have also shown that TXA2 induces a marked intrahepatic vascular constriction in a dose-dependent fashion (13). In view of this, attenuating the effects of these vasoconstrictors has been proposed as a therapeutic option for preventing the hepatic damage that occurs after stress. Studies have shown that treatment with ET-1 or TXA2 antagonists protects hepatic functions following endotoxic shock and ischemia-reperfusion of the liver (16, 18, 33, 38). However, it remains to be determined whether these vasoconstrictors play any role in producing hepatic dysfunction following trauma-hemorrhage. Furthermore, the amount of vasoconstrictors produced in the liver has not been determined after trauma-hemorrhage. The aim of this study therefore was to measure the amount of ET-1 and TXA2 produced in the liver following trauma-hemorrhage and to examine the effects of these vasoconstrictors on hepatic functions and portal circulatory resistance using the isolated liver perfusion sys-

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The isolated liver perfusion system was selected to assess the amount of vasoconstrictors released selectively from the liver. This system is preferable because it excludes the effects of decreased cardiac output and splanchnic circulation, which are observed following trauma-hemorrhage and could indirectly affect the hepatic blood flow as well as its functions.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 250–350 g were used in these experiments. 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 Alabama at Birmingham.

**Trauma-hemorrhage procedure.** The rats were fasted overnight but were allowed free access to water before the experiment. The rats were anesthetized by isoflurane inhalation (Attane, Minrad, Bethlehem, PA) followed by a 5-cm midline laparotomy as soft tissue trauma. The abdominal wound was closed in layers, and polyethylene tubes (PE-50, Becton-Dickinson, Sparks, MD) were placed in both femoral arteries and right femoral vein. The groin wounds were bathed in 1% lidocaine (Elkins-Sinn, Cherry Hill, NJ) throughout the procedure to minimize the wound pain. The rats were allowed to awake and then bled rapidly to a mean arterial pressure of 35–40 mmHg within 10 min. This hypotension was maintained until the animals could no longer maintain MAP of 35 mmHg unless extra fluid, in the form of Ringer lactate, was administered. This time was defined as maximum bleed-out time. After the maximum bleed-out, MAP was maintained between 35 and 40 mmHg until 40% of the maximum bleed-out volume was returned in the form of Ringer lactate. The rats were then resuscitated with four times the volume of the withdrawn blood over 60 min with Ringer lactate. After resuscitation, the catheters were removed and the wounds were closed. Sham-operated animals underwent the same groin incision and vessel ligation without performing the midline laparotomy or hemorrhage.

**Isolated liver perfusion.** To determine the effects of trauma-hemorrhage at different time points, studies were performed at 2 and 5 h after trauma-hemorrhage. These time points were selected on the basis of the time course of shock-induced upregulation of vasoregulators (4, 30). The rats at 5 h after sham operation were used as controls. The animals were anesthetized with isoflurane inhalation and subjected to isolated liver perfusion. The liver was exposed through a wide transverse incision, and the portal vein was isolated. Blood samples were obtained from the abdominal aorta into a heparinized syringe before the isolated liver perfusion began. Plasma was then separated by centrifugation at 3,220 × g for 12 min at 4°C and stored at −80°C until used. The bile duct was isolated from the portal vein and was cannulated with a PE-10 catheter, and the amount of bile produced was collected in preweighed tubes. The amount of bile produced was measured by collecting the bile in preweighed tubes. The portal vein was cannulated with a PE-240 catheter, and the liver was perfused with warm 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. The isolated liver perfusion was performed according to the method of Bauer et al. (3) with minor modification. Briefly, the perfusate was pumped from a reservoir in which the buffer was oxygenated by respirer (95% O2–5% CO2). The temperature of the perfusate was maintained at 36–37°C by warming the reservoir in a water bath.

After the washout period, when the effluent from the liver became clear, the thoracic venal outlet cannula was placed into the reservoir to return the perfusate flow. The flow was maintained at 30 ml/min. A pressure transducer was placed in line at the portal inlet to monitor portal pressure. The liver was perfused for 15 min with nonrecirculating perfusate and then perfused for 60 min with recirculating perfusion. The total volume of the perfusate during the recirculation perfusion was 150 ml. Small amounts of perfusate were sampled before starting the recirculation (0-min point) and after 30 and 60 min of recirculation for the measurement of lactate dehydrogenase (LDH) activity, ET-1, and thromboxane B2 (TXB2; a stable metabolite of thromboxane A2) levels.

**Plasma ALT assay.** After the plasma was separated by centrifugation, measurements for serum ALT were made spectrophotometrically using diagnostic kits from Sigma (St. Louis, MO).

**Measurement of ET-1 and TXB2 in perfusate.** Enzyme immunoassay kits were used to determine the concentration of ET-1 (Cayman Chemical, Ann Arbor, MI) and TXB2 (Assay Designs, Ann Arbor, MI), a stable metabolite of TXA2, in the perfusate. The samples were diluted 1:10 with Krebs buffer so that the highest concentration of ET-1 or TXB2 in the perfusate fell in the linear range of the standard curve. The levels of ET-1 and TXB2 were expressed in nanograms per milliliter (ng/ml) and picograms per milliliter (pg/ml), respectively.

**Histological changes after isolated liver perfusion.** At the end of isolated liver perfusion, a piece of the liver tissue was harvested for histological analysis. The samples were immersed immediately in 10% buffered formalin and stored overnight. The tissues were then 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.

**Statistical analysis.** There were six animals in each group. The results are presented as means ± SE. One-way ANOVA followed by the Student-Newman-Keuls test for multiple comparisons was used to determine the significant differences among the experimental groups. When criteria for parametric testing were violated, the appropriate nonparametric (Mann-Whitney U-test) test was used. A P value <0.05 was considered to indicate a significant difference.

**RESULTS**

**Plasma ALT levels.** After trauma-hemorrhage, plasma ALT level was substantially higher than sham at 2 h as well as at 5 h (Fig. 1A). However, the difference was statistically significant only at 5 h following trauma-hemorrhage and resuscitation. Thus hepatic damage is evident at 5 h following resuscitation.

**Portal inflow pressure.** The portal inflow pressure could be directly related to the total resistance of the intrahepatic portal circulation because of the constant flow rate used in the isolated liver perfusion system. At 2 h following trauma-hemorrhage, the level of portal inflow pressure was not significantly different compared with sham animals; however, it was significantly higher at 5 h following trauma-hemorrhage, indicating severe circulatory disturbance at that time point (Fig. 1B).

**Bile flow during the isolated liver perfusion.** In contrast to the portal inflow pressure, the bile flow during the isolated liver perfusion was the lowest at 5 h.
following trauma-hemorrhage, which was significantly less than sham values (Fig. 1C). Interestingly, when the data from all groups (2 and 5 h following trauma-hemorrhage and sham) were included, the portal in-flow pressure was inversely correlated to the amount of bile production, which was found to be significant by Pearson’s correlation analysis ($P < 0.05$; Fig. 2).

**LDH levels in perfusate.** The elevated plasma ALT levels indicate the hepatic damage before the isolated liver perfusion. However, to determine the level of damage during the isolated liver perfusion, we measured the level of LDH, a sensitive indicator of the hepatic damage in the perfusate. The level of LDH was significantly higher than sham at 2 and 5 h after trauma-hemorrhage compared with sham (Fig. 3).

**Histological change.** Tissue samples collected at the end of the isolated liver perfusion were examined to evaluate the hepatic damage. Vacular changes in the hepatocytes around the central vein, which indicates the hepatocellular damage, were most prominently observed in a group at 5 h following trauma-hemorrhage (Fig. 4). These observations further support the notion that severe circulatory derangements and tissue hypoxia are evident at that time point.

**ET-1 and TXB2 levels in perfusate.** The amount of vasoconstrictors (ET-1 and TXB2) released from the liver and accumulated in the perfusate during the isolated liver perfusion was measured by ELISA. The perfusate was collected at 0, 30, and 60 min after starting recirculating liver perfusion. The level of ET-1 in the perfusate at any time point following trauma-hemorrhage did not differ from sham levels, and it did not increase over the time course of 60 min of recirculating isolated liver perfusion. This would suggest that there is little production of ET-1 by the liver following trauma-hemorrhage (Fig. 5A). However, the level of TXB2 increased at 5 h following trauma-hemorrhage,

Fig. 1. A: plasma ALT levels at 2 and 5 h following trauma-hemorrhage (T-H) or after sham operation. Blood samples were obtained from the abdominal aorta. B: maximum portal venous pressure in isolated liver perfusion. Livers from sham or T-H animals were perfused with Krebs-Henseleit bicarbonate buffer by a flow-controlled system. Maximum pressure during 60 min of recirculating perfusion was recorded. C: total bile flow during 60 min of recirculating isolated liver perfusion. Bile was collected in preweighed tubes. Bile production is represented as $\mu$g bile/kg body wt $^{-1}$ h $^{-1}$. Data are means $\pm$ SE of 6 animals in each group. *$P < 0.05$ vs. sham by ANOVA.

Fig. 2. Correlation between portal pressure and amount of bile flow. The significance of the correlation was confirmed by Pearson’s analysis between the two parameters. BW, body wt.

Fig. 3. Lactate dehydrogenase (LDH) levels in the perfusate. Small amount of perfusate was collected at the beginning (0 min) and at 30 and 60 min during recirculating isolated liver perfusion from animals at 2 or 5 h following T-H. The assays were performed according to the manufacturer’s description. Data are means $\pm$ SE of 6 animals in each group. *$P < 0.05$ vs. sham by ANOVA.
and it was significantly higher than sham levels (Fig. 5B). TXB$_2$, a stable metabolite of TXA$_2$, progressively accumulated during the 60 min of isolated perfusion, indicating increased production of TXA$_2$ from the liver at 5 h following trauma-hemorrhage. Furthermore, a significant positive correlation between the portal pressure and the levels of TXB$_2$ and a negative correlation between the bile flow and the levels of TXB$_2$ were confirmed by Pearson’s correlation analysis (Pearson’s correlation coefficients: portal pressure vs. plasma TXB$_2$, $r = 0.624$; bile flow vs. plasma TXB$_2$, $r = -0.729$). These results indicate that the increased portal circulatory resistance and decreased bile production following trauma-hemorrhage might be associated with the increased production of TXB$_2$ from the liver but not with that of ET-1.

**DISCUSSION**

The status of the hepatic circulation is an important determinant of hepatocellular viability (46). Excessive release of vasoconstrictors in the hepatic circulation impairs hepatic microcirculation, which ultimately leads to hepatocellular hypoxia/ischemia and subsequent hepatic damage. Studies have shown that the liver produces vasoconstrictors in response to stress and that these mediators act on the hepatic circulation in an autocrine or paracrine fashion (22, 28). Among
the various vasoconstrictors, ET-1 (32, 48) and TXA2 (15, 39) have been highlighted as the most effective vasoconstrictors for the portal circulation in the liver. Administration of both vasoconstrictors through the portal vein markedly increases the portal inflow pressure in a dose-dependent fashion (6). We therefore focused on the role of ET-1 and TXA2 in the regulation of hepatic circulation following trauma-hemorrhage in this study, and our results imply that the TXA2 plays a more prominent role as an autocrine/paracrine mediator than ET-1 in producing portal hypertension following trauma-hemorrhage.

The elevated plasma ALT levels indicate that even after fluid resuscitation following trauma-hemorrhage, the hepatic damage is still progressive. We speculate that one of the reasons for this progressive hepatic damage is a sustained disturbance in hepatic circulation. This notion is supported by our observation of portal inflow pressure. At 5 h following trauma-hemorrhage, the portal inflow pressure was significantly higher compared with the sham, indicating that hepatic vascular resistance is increased at this time point. Furthermore, the bile production in the isolated liver perfusion was significantly lower than sham, suggesting that the impaired circulation deteriorates the liver integrity and function. Another interesting finding in this study is the correlation between bile flow and portal inflow pressure. Although ICG clearance, a sensitive indicator of hepatic function, was found to be decreased after trauma-hemorrhage in previous studies (42), its correlation with the portal circulatory resistance has not been investigated. The clearance of ICG, however, was not measured in this study. Nonetheless, the results indicate that circulatory disturbances following trauma-hemorrhage and resuscitation have a direct detrimental effect on the bile production. In this regard, the amount of bile production during the isolated liver perfusion has been shown to be associated with the normality of hepatic microcirculation (21, 42).

The impaired hepatic circulation at 5 h following trauma-hemorrhage was further confirmed by evaluating the histological samples from the liver obtained after the isolated liver perfusion. In contrast to the normal hepatic tissue obtained from sham and trauma-hemorrhage animals at 2 h, the liver from trauma-hemorrhage at 5 h contained damaged cells around the central veins. These areas are more vulnerable to the decreased blood flow and hypoxia because of the oxygen gradient along the sinusoid. These results lead us to conclude that the vasoconstriction in the portal venous system is more severe following trauma-hemorrhage at 5 h after resuscitation.

To determine which vasoconstrictor was responsible for producing the circulatory alterations in the portal venous system, we measured the release of ET-1 or TXB2 into the perfusate by ELISA. ET-1 production from the liver was not affected in our model of trauma-hemorrhage and resuscitation. Studies reported an important role of ET-1 as a systemic or organ vasoconstrictor after hemorrhagic shock (35). Furthermore, plasma ET-1 levels have been reported to increase immediately following hemorrhagic shock in canine models (9). However, there is no available report on the production of ET-1 in the liver and the autocrine or paracrine effects of ET-1 in the portal system under those conditions. From the results of our studies, we propose that the autocrine or paracrine effects of ET-1 on the hepatic portal circulation are minimal, if any, following trauma-hemorrhage. It should be pointed out, however, that the impaired portal circulation and hepatic damage observed in this study could be due to the enhanced sensitivity of the liver to ET-1 even if the amount of the circulating ET-1 is not altered. In this regard, Pannen et al. (31) reported that the hepatic perfusion response to ET-1 was enhanced after hemorrhagic shock, which leads to greater increases in portal resistance, decreases in sinusoidal diameters, and decreased hepatic oxygen delivery compared with sham. This might be due to the altered expression of endothelin receptor subtype as shown in other hepatic stress models (45, 46). However, the study of Pannen et al. (31) used exogenous ET-1 to test its vasoconstrictive effects on the hepatic circulation and actual level of ET-1 in the portal circulation, and its effect on producing the portal hypertension remains unknown. Nonetheless, further study should be performed to elucidate whether the increased constricor response to ET-1...
contributes to the hepatic damage even if the amount of circulating ET-1 is not changed.

In contrast to the measured ET-1 levels in the perfusate, TXB₂ was markedly elevated at 5 h following trauma-hemorrhage and resuscitation. This was associated with increased portal pressure and decreased bile production. Furthermore, the levels of plasma ALT and perfusate LDH (data not shown) showed high levels at this time point. As reported previously (7, 10), the increased resistance of the liver in isolated liver perfusion is closely correlated with the sinusoidal or presinusoidal microcirculatory failure and subsequent hepatic damage. Therefore, these results suggest an important role of TXA₂ in controlling the portal resistance and its contribution to the hepatic damage following trauma-hemorrhage and resuscitation.

Our previous studies (24) have shown that hepatic damage occurs not only at 24 h following trauma-hemorrhage but also at an early time point (2). Because the present study was limited to 5 h following trauma-hemorrhage, it remains unknown whether TXB₂ release from the liver is sustained at 24 h following trauma-hemorrhage. However, we speculate that an inflammatory response, instead of microcirculatory failure, in the liver is more important for the hepatic damage at 24 h after trauma-hemorrhage. This speculation is supported by our previous observations (1), which demonstrated elevated IL-6 and TNF-α production by the Kupffer cells in the liver at 24 h following trauma-hemorrhage.

It could be argued that the results seen from isolated livers have potential limitations and cannot be extrapolated to in vivo conditions. The spleen and intestine have been identified as important sources of vasoconstrictors such as ET-1 and TXA₂ under stressful conditions (25, 26). Therefore, the levels of these vasoconstrictors could be increased in the splenic vein or superior mesenteric vein following trauma-hemorrhage. Furthermore, it is possible that persistent action of these vasoconstrictors exerted in vivo continuously stimulated the hepatic portal vein over the course of 60 min of isolated perfusion. Whereas this is a possibility and must be clarified by other methodology, we believe that the effects of the above-mentioned agents/mechanisms are minimum, because the liver was thoroughly washed for at least 15 min by nonrecirculating perfusion that should have markedly lowered the effects of these vasoconstrictors. Furthermore, the use of the isolated liver system permits us to exclude the effects of unstable systemic hemodynamics following trauma-hemorrhage on the hepatic circulation. Moreover, measurement of vasoconstrictors, which are solely released by the liver, is possible in this system. Both of the above aspects are not possible to determine in the in vivo conditions.

The results of the present study have provided us with a rationale to consider a TXA₂ inhibitor as a potential therapeutic option in preventing the hepatic damage after trauma-hemorrhage. In this regard, selective thromboxane synthetase inhibitors have been shown to be useful in preventing cerebral vasospasms and delayed cerebral ischemic symptoms after subarachnoid hemorrhage due to aneurysmal rupture (34, 37). TXA₂ synthesis inhibitors have also been reported to minimize hepatic damage in different hepatic stress models such as total liver ischemia (19), bile duct ligation (12), and hepatectomy (36). Treatment of rats with specific TXA₂ synthesis inhibitors following hemorrhagic shock exhibited significantly longer survival times than rats receiving vehicle (8). The beneficial effects of this drug are observed from the attenuation of the TXA₂-related vasoconstriction. It is therefore possible that treatment with TXA₂ synthesis inhibitors or specific TXA₂-receptor antagonists following trauma-hemorrhage may ameliorate vasospasm in the portal circulation, which could improve the hepatic microcirculation and prevent hepatic damage under those conditions.

In summary, this study demonstrated increased portal resistance and decreased bile production occurring with hepatic damage as indicated by increased plasma ALT levels and histological changes at 5 h following trauma-hemorrhage. The level of TXA₂ metabolite produced by the liver peaked at this time point, thus indicating an important role of TXA₂ producing hepatic damage in this model.

DISCLOSURES

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REFERENCES


