Insights into the role of interleukin-6 in the induction of hepatic injury after trauma-hemorrhagic shock

Balazs Toth, Yukihiro Yokoyama, Martin G. Schwacha, Richard L. George, Loring W. Rue III, Kirby I. Bland, and Irshad H. Chaudry

Center for Surgical Research and Department of Surgery, University of Alabama at Birmingham, Birmingham, Alabama 35294

Submitted 10 May 2004; accepted in final form 3 August 2004

Toth, Balazs, Yukihiro Yokoyama, Martin G. Schwacha, Richard L. George, Loring W. Rue III, Kirby I. Bland, and Irshad H. Chaudry. Insights into the role of interleukin-6 in the induction of hepatic injury after trauma-hemorrhagic shock. J Appl Physiol 97: 2184–2189, 2004.—Although systemic interleukin-6 (IL-6) is a cytokine; liver; hemorrhage; lactate dehydrogenase; isolated liver perfusion

TRAOA AND HEMORRHAGIC SHOCK remain significant clinical problems. Furthermore, the patients who survive a major traumatic injury have a high risk for developing subsequent sepsis, multiple organ failure, and death (13). Experimental results show that cell and organ functions are depressed after trauma-hemorrhage. In this regard, hepatocellular function is depressed and microvascular blood flow as well as cardiac output are not maintained after trauma-hemorrhage despite fluid resuscitation (24). Furthermore, it has been shown that immune functions are affected as well: specific and nonspecific immune responses are impaired causing increased susceptibility to sepsis after trauma and hemorrhagic shock (1).

Several animal and clinical studies indicate the importance of the proinflammatory cytokine interleukin (IL)-6 after trauma-hemorrhage (2). Elevated IL-6 levels have been correlated with decreased organ function in experimental settings and increased lethality in clinical studies (8, 9, 21). These results therefore suggest that measurement of circulating IL-6 levels after trauma-hemorrhage might be predictive of patient prognosis. Studies by Remick et al. (23) indicated that elevated IL-6 levels at 6 h after the induction of sepsis is predictive of subsequent mortality. Nonetheless, it remains unknown whether IL-6 is only a marker of injury or whether IL-6 also plays an important role in inducing organ dysfunction and mortality after major injury. The pathophysiology of hepatocellular dysfunction and liver damage after trauma-hemorrhage is multifactorial; however, increased production of IL-6 may be an important contributor in the development of the hepatocellular injury and the subsequent liver damage under those conditions (13). Studies have also suggested that IL-6 is a proinflammatory cytokine under such conditions (14, 16). However, the final effect of IL-6 on the inflammatory response is a result of the sum of two countereffective mechanisms: a paracrine, inflammation-promoting effect and a systemic, endocrine-type effect of diminishing inflammation (16). Moreover, one of the major sources of IL-6 production after trauma-hemorrhage is the liver (18, 34). In this regard, the vascular endothelial cells and perivascular nonparenchymal cells are specifically activated in the liver after massive hemorrhage to produce inflammatory cytokines, such as IL-6 and tumor necrosis factor-α (TNF-α) (32, 33). Previous studies have suggested that IL-6 produces circulatory effects, possibly through the thromboxane A2 (TxA2) pathway (5). TxA2 is a potent vasoconstrictor that is capable of increasing portal venous resistance in both a paracrine and autocrine fashion. In addition, recent findings from our laboratory have implicated thromboxane in the development of liver dysfunction after trauma-hemorrhage (33). In view of this information, we hypothesized that elevated IL-6 after trauma-hemorrhage contributes to the development liver injury under those conditions.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (275–300 g, Charles River Laboratories, Wilmington, MA) were used in this study. All experiments were performed in adherence to the National Institutes of Health guidelines for the use of experimental animals and approved.
by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

**Experimental procedures.** A nonheparinized model of trauma-hemorrhage in the rat was used in this study as described in detail elsewhere (30, 31). Briefly, male Sprague-Dawley rats were fasted overnight before the experiment, but they were allowed water ad libitum. The rats were anesthetized by isoflurane (Attane, Mallinckrodt Veterinary, Mundelein, IL) inhalation before the induction of soft tissue trauma (i.e., 5-cm midline laparotomy). The abdomen was then closed in layers, and catheters were placed in both femoral arteries and the right femoral vein [polyethylene (PE)-50 tubing; Becton-Dickinson, Sparks, MD]. The wounds were bathed with 1% lidocaine (Elkins-Sinn, Cherry Hill, NJ) throughout the surgical procedure to minimize postoperative pain. The rats were then allowed to awaken, after which they were bled to a mean arterial pressure (MAP) of 35 mmHg within 10 min. Rapid bleeding places the animals in a depressed state of sensibility, thus minimizing the stress to them. The time at which the animals could no longer maintain a MAP of 35 mmHg without extra fluid was defined as maximum blood out. The rats were then maintained at this MAP until 40% of the shed blood was returned in the form of Ringer lactate. The animals were then resuscitated with four times the volume of withdrawn blood with Ringer lactate over 60 min. At the end of resuscitation, the catheters were removed, the vessels were ligated, and skin incisions were closed with sutures. Sham-operated animals underwent the same groin dissection, which included the ligation of the femoral artery and vein; however, neither trauma-hemorrhage nor resuscitation was carried out. At 2, 5, or 24 h after trauma-hemorrhage and resuscitation, the rats were anesthetized, and livers were isolated and perfused as described below. In an additional set of animals, treatment with anti-IL-6 (16.6 \(\mu\)g/kg body wt) or 10 \(\mu\)g/kg body wt immunoglobulin G (IgG) (vehicle) via infraperitoneal injection at the onset of resuscitation was carried out. The livers were then isolated and perfused at 5 h after resuscitation. The dose of anti-IL-6 (16.6 \(\mu\)g/kg body wt) used in these experiments was based on the work of Tuna et al. (27) in a rat model studying blood flow in the central nervous system. The animals were then returned to their cages and were allowed food and water ad libitum until death. Because the model of trauma-hemorrhage used in this study was severe, we did observe a mortality rate of 39% at ~24 h after resuscitation. The animals that died were not included in the study.

**Isolated liver perfusion.** The isolated liver perfusion model was performed as described previously with minor modification (33). The liver was exposed through a wide transverse incision, and the portal vein was isolated. The bile duct was cannulated with a PE-10 tube, and the bile was collected in preweighted tubes for 15 min throughout the experiment. Before cannulation of the portal vein, blood samples were drawn through aortic puncture. After cannulation of the portal vein with a PE-240 catheter, the liver was perfused with Krebs-Henseleit bicarbonate buffer (in mM: 118 NaCl, 4.7 KCl, 1.2 MgSO\(_4\), 1.2 KH\(_2\)PO\(_4\), 25 NaHCO\(_3\), 0.1 EDTA, and 2.5 CaCl\(_2\); pH 7.4) for 10 min to wash out the blood. The isolated liver perfusion was performed using a constant flow rate (100 ml·min\(^{-1}\)·kg body wt\(^{-1}\)). The warmest perfusate was pumped from a reservoir into an overflow chamber and oxygenated via an elastic tubing oxygenator (95% O\(_2\)-5% CO\(_2\)). The temperature of the perfusate was maintained at 36–37°C by warming the reservoir in a water bath. A low-pressure analyzer was placed in line immediately before the portal inlet cannula. All data were acquired using DigiMed Systems Analyzers LPA-200 (Micro-Med, Louisville, KY). The LPA-200 had a sensitivity of 0.0 ± 0.2 mmHg with a sampling and display rate of 0.5 s. The system was calibrated by using a mercury pressure manometer. The liver was perfused for 60 min with a recirculating system. The total volume of perfusate during recirculating perfusion was 150 ml. The perfusate was sampled before the start of recirculation (0 min) and every 15 min of recirculation for the measurement of thromboxane B\(_2\) (TxB\(_2\)), endothelin-1 (ET-1), IL-6, and lactic dehydrogenase (LDH) levels. Portal pressure was monitored and recorded throughout the experiment.

**Enzyme immunoassay for TxB\(_2\) and ET-1.** Enzyme immunoassay kits for 11-dehydro-TxB\(_2\) (Cayman Chemicals, Ann Arbor, MI) and rat ET-1 (Assay Design, Ann Arbor, MI) were used to determine the concentration of these mediators in the perfusate and plasma. TxA\(_2\) is rapidly hydrolyzed, nonenzymatically, to form TxB\(_2\); therefore, assay cross-reactivity is not problematic. The levels of TxB\(_2\) and ET-1 were expressed as picograms per milliliter.

**Alanine aminotransferase and LDH assays.** Blood samples obtained from the abdominal aorta of trauma-hemorrhage and sham rats and perfuse samples were examined for alanine aminotransferase (ALT) and LDH levels. Measurements were performed spectrophotometrically by using diagnostic kits from Sigma (St. Louis, MO) according to the manufacturer’s descriptions.

**Determination of cardiac output and blood flow.** The microspheres method described earlier by Ba et al. (4) was used. Briefly, at 5 h after the end of the resuscitation, animals were anesthetized with isoflurane. The right femoral artery was catheterized with PE-50 tubing, and the catheter was introduced into the left ventricle. The position of the catheter tip in the left ventricle was confirmed by the ventricular pulse pressure. Stroncum-R5-labeled microspheres (15 \(\mu\)m in diameter) were suspended in 10% dextran containing 0.05% Tween 80 to prevent aggregation. The microspheres were dispersed by vortexing for 3 min before infusion. A quantity of 0.2–0.25 ml of suspension with an activity of ~2–2.5 \(\mu\)Ci (~500,000 counts/min) per rat was infused into the left ventricle over 20 s at a constant rate. Approximately 150,000 microspheres were injected into each animal. The reference blood sample was withdrawn from the right femoral artery starting 10 s before the infusion, and sampling continued for 80 s at a constant rate of 0.7 ml/min. Immediately after the microsphere infusion was completed, normal saline solution was infused through the left ventricular catheter (twice the volume of the withdrawn blood). At the end of the measurement, the animals were killed with intravenous lidocaine injection. Organs were removed, weighed, and placed into counting vials. The radioactivity was counted on a 1740 Wizard gamma counter (Wallac, Turku, Finland). The reference blood sample was transferred to a vial and counted as well. Cardiac output and blood flow were calculated as previously described (29). Portal flow was calculated as the sum of the blood flow of the stomach, spleen, pancreas, small and large intestine (including the upper part of the rectum), and mesentery. Total hepatic blood flow was calculated as the sum of portal blood flow and hepatic arterial blood flow (29).

**Determination of the potential vascular effects of IL-6 and thromboxane.** In a different set of experiments, normal animals underwent isolated liver perfusion as described above. The first group of animals received two different dosages of IL-6 (R&D Systems, Minneapolis, MN): 400 \(\mu\)g/ml and 4,000 \(\mu\)g/ml. The dose of IL-6 used in these experiments represents an ~100,000-fold excess of that observed in the circulation after trauma-hemorrhage. The second group received two different dosages of carbocyclic TxA\(_2\) [1 nmol/150 ml (6.7 nm) and 10 nmol/150 ml (67 nm), Cayman Chemical], a stable TxA\(_2\) agonist. The concentrations of carbocyclic TxA\(_2\) employed were on the basis of previous studies showing perfusate level of TxB\(_2\) of ~0.3 nM (33) and concentrations required to exert a biological effect.

**Statistical analysis.** Regression analysis was performed using SAS software. Data are presented as means ± SE. Statistical differences between groups were determined by ANOVA followed by Student-Newman-Keuls post hoc test or ANOVA on ranks followed by Dunn’s test, and the differences were considered significant if \(P < 0.05\).

**RESULTS**

**Relationship between IL-6 and liver injury.** Trauma and hemorrhagic shock increased plasma levels of the liver enzyme ALT, a marker of liver injury (Fig. 1A). Similarly, an increase
in circulating plasma levels of IL-6 was also observed (Fig. 1B). A significant correlation was observed between plasma IL-6 and ALT levels after trauma-hemorrhage ($R^2 = 0.440$; Fig 1C). In the isolated perfused liver preparation, trauma-hemorrhage induced increased levels of LDH in the perfusate at 60 min after the initiation of perfusion (Fig. 2A). A similar increase in perfusate IL-6 levels appeared (Fig. 2B) that positively correlated LDH perfusate levels ($R^2 = 0.367$; Fig. 2C).

**Effects of IL-6 blockade on liver injury and function.** Although trauma-hemorrhage increased portal pressure compared with sham animals, the differences did not reach statistical significance (Fig. 3). Blockade of IL-6 did not change the portal pressure. Trauma and hemorrhagic shock significantly increased the circulating levels of ALT 5 h after resuscitation compared with sham animals (Fig. 4A). This trauma-hemorrhage-induced increase in plasma ALT levels was significantly attenuated by treatment with anti-IL-6 (Fig. 4A), indicating decreased hemorrhage-induced liver damage. Circulating levels of the vasoactive mediator TxB2 were elevated after trauma-hemorrhage compared with shams (Fig. 4B). Anti-IL-6 treatment reduced TxB2 levels by ~40% compared with trauma-hemorrhage rats treated with IgG. Trauma-hemorrhage significantly decreased bile flow 5 h after the end of resuscitation (Fig. 4C). Anti-IL-6 treatment did not alter the bile production in sham animals; however, the antibody treatment in the trauma-hemorrhage rats restored bile production to sham levels.

---

**Fig. 1.** Effect of trauma-hemorrhage (T-H) on plasma alanine aminotransferase (ALT; A), and interleukin-6 (IL-6; B) levels. C: correlation between ALT and IL-6 levels. Plasma samples were obtained from sham animals and T-H animals at 2, 5, and 24 h after resuscitation, and ALT and IL-6 levels were determined as described in MATERIALS AND METHODS. Values are means ± SE of 4–6 animals in each group. *P < 0.05 compared with sham. # P < 0.05 compared with 2 h. † P < 0.05 compared with 5 h.

**Fig. 2.** Effect of T-H on perfusate lactate dehydrogenase (LDH; A) and IL-6 (B) levels. C: correlation between LDH and IL-6 levels. Livers were isolated and perfused from sham animals and T-H animals at 2, 5, and 24 h after resuscitation. The isolated liver perfusion was performed as described in MATERIALS AND METHODS. Perfusate samples were collected after 60 min of perfusion. Values are means ± SE of 4–6 animals in each group. *P < 0.05 compared with sham.

**Fig. 3.** Effect of IL-6 neutralization on T-H-induced changes in portal pressure. Rats were subjected to sham treatment or T-H and were treated with either immunoglobulin G (IgG) or anti-IL-6 as described in MATERIALS AND METHODS. At 5 h after resuscitation, livers were isolated and perfused and portal pressure measured. □, Sham treated with IgG; ◊, sham treated with anti-IL-6; ○, T-H treated IgG; ●, T-H treated with anti-IL-6. Values are means ± SE of 6 animals in each group.
There was no difference in portal blood flow between IgG- and anti-IL-6 sham-treated animals. Trauma and hemorrhage caused a significant decrease in the liver perfusion rate in the IgG-treated group. Whereas anti-IL-6 treatment significantly increased hepatic artery blood flow after trauma-hemorrhage (Fig. 5B), it had no effect on portal venous flow under such conditions (Fig. 5C).

**Effect of IL-6 and TxA2 on portal pressure.** Normal animals were treated with two different concentrations of IL-6 (400 and 4,000 μg/ml). IL-6 treatment was ineffective in altering portal pressure (Fig. 6). To test the possible effects of thromboxane on the liver circulation, normal animals were treated with carbocyclic TxA2 (1 nmol/150 ml and 10 nmol/150 ml). Significant dose-dependent increases in portal pressure were observed after infusion of carbocyclic TxA2 (Fig. 7).

**DISCUSSION**

Trauma-hemorrhage continues to be one of the most common causes of mortality in surgical intensive care units (13). Furthermore, trauma-hemorrhage is the leading cause of death for persons under the age of 40 yr in the Western society (22). Trauma and the subsequent hemorrhagic shock lead to the activation of a series of mediator systems, such as the cytokine cascade, neutrophil activation, and other mechanisms, which can contribute to the tissue damage and subsequent organ failure (6, 28). In this study, we examined whether IL-6 plays any role in inducing liver dysfunction and damage after trauma-hemorrhage. Our findings indicate that IL-6 is not only a marker of liver injury after trauma-hemorrhage but that it also plays a significant role in the induction of hepatic injury and dysfunction.

IL-6 is a multifunctional cytokine acting on various cell types (25, 26), and it plays an important role in the mainte-
Carbocyclic thromboxane A2 was added at the indicated times. Values are normal rats were isolated and perfused, and portal pressure was determined. Representative results from 3 animals.

nance of homeostasis. Perturbations in its production are observed after pathophysiological conditions such as trauma-hemorrhage and sepsis. Additionally, IL-6, together with TNF-α and IL-1, induces the acute-phase response, which consists of fever, increased corticosteroid, and acute-phase protein release (26). IL-6 is considered a proinflammatory cytokine, and the enhanced release of the inflammatory cytokines TNF-α, IL-1β, and IL-6 are the hallmark of hemorrhagic shock and sepsis (13). In addition to its role in inflammation, IL-6 induces the differentiation and development of B and T cells, myeloid cells, megakaryocytes, osteoclasts, and hepatocytes and it acts as a growth factor for renal cell carcinoma and Kaposi’s sarcoma and promotes the growth of hematopoietic stem cells (26). IL-6 is also considered an important factor in liver regeneration, contributing to hepatocyte proliferation. Because a soluble, agonistic IL-6 receptor exists, virtually all cells can be IL-6 responsive (16). It has been previously demonstrated that both the IL-6 mRNA and protein are produced in the livers, lungs, and intestines of rats after trauma-hemorrhage (11, 12). In this regard, local administration of IL-6 in the lungs of normal rats increased polymorphonuclear cells infiltration and lung damage (12). Furthermore, higher circulating levels of IL-6 were demonstrated in animals and humans after trauma-hemorrhage (6, 10, 19). In contrast, systemic infusion of IL-6 after trauma and hemorrhagic shock reduced inflammation and injury in the liver and lung (15). Thus it may be concluded that IL-6 has two different effects: a local proinflammatory and a systemic anti-inflammatory effect (16, 18).

It is well established that the liver is a major target for organ injury in low flow states, such as trauma-hemorrhage. The decrease in cardiac output after trauma-hemorrhage is associated with a disproportionately greater decrease in hepatic blood flow, resulting in insufficient oxygen delivery to the liver and thus causing hepatocellular hypoxia (20). This hypoxic insult is associated with a significantly increased production and release of proinflammatory cytokines, such as IL-6, TNF-α, and IL-1. It has been shown that circulating IL-6 concentrations are elevated after trauma-hemorrhage (3). Furthermore, it has been shown that a positive correlation exists between plasma IL-6 levels and the outcome after sepsis (23). Our data indicate that there is a positive correlation between the circulating IL-6 levels and hepatic damage after trauma-hemorrhage, measured by ALT levels after trauma-hemorrhage. Nonetheless, although these experiments established an association between elevated plasma levels of IL-6 and liver damage, they did not establish a causal relationship. Because our results showed a significant decrease in the circulating levels of TxB2 (a stable degradation product of TXA2) after the blockade of IL-6, we hypothesized that the observed correlation between the IL-6 levels and liver damage might due to TXA2-mediated decrease in perfusion. Despite the slightly higher portal pressure values in the IL-6 blockade group, we could not detect significant differences on the macrocirculatory level using the isolated liver perfusion model. The microsphere studies, which were carried out to determine the role of IL-6 in liver microcirculation, revealed that the effect of this cytokine was more pronounced on the arteries of the liver and that IL-6 blockade did not produce any significant changes in the venous blood flow. Studies have also suggested that IL-6 produces direct circulatory effects (5). At the microcirculatory level, IL-6 can cause vasoconstriction, possibly through the TXA2 pathway (5). In contrast, IL-6 has been reported to cause vasodilatation in vivo, although IL-6 was ineffective in causing relaxation of similar arterioles under isolated in vitro conditions (17). The direct administration of IL-6 in our system did not produce any significant changes in liver circulation. Even though the doses of IL-6 employed in these studies greatly exceeded those observed in vivo, no effect was observed on liver circulation. In contrast, the administration of carbocyclic TXA2, a stable TXA2 analog, resulted in a dose-dependent increase in portal pressure. Although the concentrations of TXA2 used were ~10- to 100-fold higher than those previously observed in our laboratory’s model (33), they still support the concept that IL-6 is mediating liver injury via thromboxane-mediated pathway. Our laboratory’s previous studies have shown that thromboxanes play a central role in the development of portal hypertension after trauma-hemorrhage (33).

Previous studies have also shown that administration of TNF-α at a dose that did not affect cardiac output or hepatic microcirculation produced a marked increase in plasma IL-6 levels and depressed active hepatocellular function (29). Liver enzymes were not measured in that study, and thus it remains unknown whether the dose of TNF-α that does not affect cardiac output and hepatic blood flow also produces hepatic damage via upregulation of IL-6 production. Irrespective of that, the fact remains that our laboratory’s previous study also indicated that IL-6 is responsible for producing hepatic abnormality (13a).

It should be noted, however, that there are some limitations of our study. For instance, the isolated liver perfusion model does not completely simulate in vivo conditions. In view of that, the actual local IL-6 release is not known in vivo and should be determined. Furthermore, the thromboxane pathway seems to be downstream of the action of the circulatory effects of IL-6. Despite the fact that we could show a decrease in the TxB2 levels after anti-IL-6 treatment after trauma-hemorrhage, the exact relationship between the two agents remains to be completely elucidated. Additionally, the exact cellular location of action for IL-6 and thromboxane remains to be determined. Elucidation of the mechanisms of action of IL-6 after trauma-hemorrhage holds promise in the development of specific therapeutic approaches that will limit hepatic injury and reduce patient morbidity and mortality under those conditions.

Fig. 7. Effect of carbocyclic thromboxane A2 on portal pressure. Livers of normal rats were isolated and perfused, and portal pressure was determined. Carbocyclic thromboxane A2 was added at the indicated times. Values are representative results from 3 animals.
REFERENCES


