Trauma- and Sepsis-Induced Hepatic Ischemia and Reperfusion Injury
Role of Angiotensin II

Hypothesis: We hypothesized that angiotensin II, a potent vasoconstrictor, is involved in the occurrence of hepatic ischemia after burn and sepsis, and that administration of angiotensin II antagonist DuP753 would ameliorate this process.

Design: Randomized animal study.

Setting: University laboratory, investigational intensive care unit, University of Texas Medical Branch, Galveston.

Materials: Female pigs (n=18, weighing 20-25 kg).

Interventions: All animals were prepared with ultrasonic flow probes on the portal vein and the common hepatic artery. Catheters were inserted in the superior mesenteric and left hepatic veins. After 5 days all animals were anesthetized and 12 of them received 40% total body surface area third-degree burn. Escherichia coli lipopolysaccharide (100 µg/kg) was intravenously administered at 18 hours postburn DuP753 was administered intravenously in a dose of 1 µg/kg to 6 pigs immediately after the burn. All animals were studied for 42 hours.

Main Outcome Measures: Systemic and hepatic hemodynamics were measured and blood samples were drawn for determinations of arterial, mixed venous, and portal blood gases at baseline and at 14 consecutive time points, starting 1 hour after the burn.

Results: Burn caused a 4.6-fold increase in hepatic arterial vascular resistance and a 49% decrease in hepatic arterial blood flow. Postburn administration of angiotensin II receptor blocker DuP753 yielded a significant improvement in the hepatic arterial hemodynamics (only 12% increase in hepatic arterial vascular resistance and 8% decrease in hepatic arterial blood flow, P<.05 vs nontreated group, analysis of variance [ANOVA]). Postlipopolysaccharide hepatic arterial blood flow was significantly reduced (12% of baseline, P<.05, ANOVA), in contrast to DuP753-treated animals (64% of baseline, P<.05 vs nontreated group, ANOVA). Postburn blocking of angiotensin II receptors yielded a significant improvement in postlipopolysaccharide portal venous blood flow (85% of baseline vs 48% of baseline in nontreated animals, P<.05, ANOVA). Postburn endotoxemia resulted in a significant decrease of hepatic oxygen delivery (22% of baseline) and hepatic oxygen consumption (30% of baseline), while no marked changes were observed in the DuP753-treated group (P<.05 vs nontreated group, ANOVA).

Conclusions: Angiotensin II seems to play a pivotal role in burn- and sepsis-induced hepatic ischemia and reperfusion injury. Blocking angiotensin II receptors by DuP753 seems to abrogate this adverse effect of thermal injuries and sepsis on hepatic perfusion and oxygenation.

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Although sepsis and multiple organ dysfunction syndrome are responsible for 50% to 80% of all surgical intensive care unit deaths, available treatment regimens are mainly supportive and the underlying mechanisms of these syndromes remain to be defined.1,2

It has been postulated that the amplified reaction of the primed inflammatory response system of burn patients to a subsequent insult, initiated by bacteria and their by-products (endotoxins), is responsible for the typical pathophysiological alterations, seen in postinjury sepsis.2

As the liver seems to have a gate function for endogenous bacteria and their endotoxins, it can be argued that impairment of this hepatic clearance function may potentiate systemic effects of gut-barrier failure by allowing indigenous bacteria or endotoxin to reach the systemic circulation, where they potentiate systemic inflammatory responses.3

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MATERIALS AND METHODS

The following experimental protocols were approved by the Animal Care and Use Committee of the University of Texas Medical Branch, Galveston (approved protocol ACUC 90-09-103). Eighteen female mini-pigs, weighing between 20 and 25 kg, were prepared surgically 3 days before the experiment. After an overnight fast, the pigs were anesthetized with intramuscular ketamine hydrochloride (10 mg/kg) and mechanically ventilated with 2% to 2.5% halothane after endotracheal intubation. A subcostal incision was performed. Transit time ultrasonic flow probes (4-10 mm; Transonic Systems Inc, Ithaca, NY) were placed on the common hepatic artery and the portal vein. Catheters (6.5F) were positioned in the superior mesenteric vein on the common hepatic artery and the portal vein. Catheters (model 93 A-131-5F; American Edwards Laboratories, Anasco, Puerto Rico) was positioned in the pulmonary artery through the right jugular vein. A 12F Foley catheter was inserted in the urinary bladder.

After surgery, the animals were kept in recovery slings for 24 hours, then placed in runs for 5 days with free access to food and water. On the day of the experiment, the animals were reanesthetized and, through a neck incision, an arterial catheter was placed via the right common carotid artery into the abdominal aorta. A Swan-Ganz thermal dilution catheter (model 93 A-131-5F; American Edwards Laboratories, Anasco, Puerto Rico) was positioned in the pulmonary artery through the right jugular vein. A 12F Foley catheter was inserted in the urinary bladder.

The animals were kept in slings for monitoring. Throughout the study, all animals received enteral feeding (Osmolite) at 25 mL/h and nothing orally. Baseline data were collected after complete recovery from anesthesia.

Pigs were randomized into the following 3 groups:
1. Burn/lipopolysaccharide-treated (LPS) group (n=6) had a 40% total body surface area (TBSA) third-degree flame burn under general anesthesia, as described elsewhere. The pigs were resuscitated according to the Parkland formula and received lactated Ringer solution, 4 mL/kg per percentage of TBSA burn, starting immediately after the burn, half of which was given in the first 8 hours after the burn and the remainder in the following 16 hours. Eighteen hours after the burn, 100 µg/kg of Escherichia coli LPS (O111:B4; Difco, Detroit, Mich) was intravenously administered. During the second day of the experiment, burned animals received lactated Ringer solution at 3.5 mL/m²/burned area and 2 mL/kg per hour for daily maintenance.
2. Sham group (n=6) had a sham burn under anesthesia. Eighteen hours later the animals received the diluent (0.9% sodium chloride) used for the endotoxin. Lactated Ringer solution was administered at a rate of 2 mL/kg per hour for daily maintenance.
3. Treatment group (n=6) underwent the same procedure as in the burn/LPS group, except for the administration of angiotensin II inhibitor (DuP753; DuPont Merck, Wilmington, Del) intravenously in a dose of 1 µg/kg, immediately after burn.

The results of our previous studies in a porcine model indicated that thermal injury has a selective vasoconstrictive effect on the hepatic arterial circulation, yielding hepatic ischemia and a reduction of hepatic oxygen delivery. Furthermore, a second insult (endotoxemia) was shown to cause a pronounced hepatic ischemia/reperfusion injury, associated with an inadequate hepatic oxygen delivery (hDO₂) and a pathologic supply-dependent hepatic O₂ consumption (hVO₂). In this study an attempt was made to investi-
gate the role of angiotensin II as a possible mediator involved in this process.

RESULTS

SYSTEMIC HEMODYNAMICS

Baseline hemodynamic measurements were similar in all groups. All animals survived the study period. Throughout the experiment, sham animals maintained their systemic (Figure 1 and Figure 2) and hepatic (Figure 3 and Figure 4) hemodynamics within baseline range.

After thermal injury, CO showed a slight increase during the first 6 hours, returning to baseline 8 hours after the burn (Figure 1). This increase was associated with a concomitant fall in the SVRI, whereas SVRI decreased to 78% of baseline level (Figure 1). No significant differences were noted in MAP and CVP between the 3 groups (Figure 2).

Following administration of LPS, a typical biphasic response was observed. The hemodynamic alteration was more pronounced during the second phase, as after a marked drop of CO to 77% of baseline level a hyperdynamic period began to be manifest 8 hours after endotoxin (Figure 1). At this time point, SVRI dropped to 69% of baseline. Mean arterial pressure showed a 14% decrease, immediately after LPS infusion in both burn/LPS groups (Figure 2). During the further post–LPS course, no significant differences were noticed between groups in MAP and CVP. DuP753 treatment ameliorated to a certain extent the alteration in systemic circulation, occurring after burn and LPS administration (Figure 1 and Figure 2).

HEPATIC HEMODYNAMICS

Hepatic Arterial Circulation

Hepatic arterial blood flow decreased significantly to approximately half of baseline level during the first 4 hours after the burn (Figure 3). This fall in HABF was associated with a 4.6-fold increase in HAVR, as early as 1 hour after the burn (Figure 4).

As compared with burned animals, not receiving the angiotensin II antagonist, DuP753-treated animals showed a slight increase of HAVR (12% of baseline value) follow-
ing thermal insult (Figure 4). Animals in the DuP753 treatment group maintained, in contrast with the nontreated burned animals, a stable HABF near baseline with an average fall of 8% of baseline (Figure 3). Heparic arterial hemodynamic measurements recovered completely in all animals to baseline values at 18 hours after the burn.

Administration of LPS to burned animals resulted in a biphasic response of hepatic ischemia and reperfusion. The second insult resulted in a significant hepatic arterial vasoconstriction with a 6-fold increase of HAVR during the first hour after LPS administration.

Correspondingly, HABF decreased significantly to 12% of baseline during the same time of maximum increase of HAVR (Figure 3 and Figure 4). After an initial recovery of HABF to baseline values, 6 hours after LPS administration, a marked elevation (120%-152% of baseline) was noticed during the following 6 hours.

Burned animals, treated with DuP753, showed a transient drop HABF to 64% of baseline during the first 30 minutes after the second insult (LPS). DuP753 treatment attenuated significantly the hepatic arterial vasoconstriction, caused by the administration of LPS to burned animals, as HAVR only increased to 178% of baseline. The post-LPS vasoconstrictive period was very short (30 minutes), where after both HAVR and HABF measurements were within baseline range (Figure 3 and Figure 4).

**Hepatic Portal Circulation**

After an initial decrease (86.5% of baseline) in PVBF during the first 1 hour after the burn, PVBF began to increase, reaching 135% of baseline at 4 hours after the burn (Figure 5). The increased PVBF was associated with a moderate decrease in HPVR (65%-83% of baseline values) during the same period (Figure 6). Portal venous pressure showed a 40% increase 1 hour postburn and declined thereafter to 120% of baseline level at 4 hours postburn (Figure 7). In contrast, animals treated with DuP753 did not show any marked increase in their postburn PVBF (Figure 5). Hepatic arterial vascular resistance in the treatment group decreased to 63% of baseline value during the first 4 hours after burn (Figure 6). A 20% decrease in PVP was observed in DuP753-treated animals during the early postburn phase (Figure 7).

Similar to the hepatic hemodynamic arterial variables, portal hemodynamic measurements showed the same pattern of recovery to baseline values at 18 hours postburn in all animals (Figures 5-7).

The second insult yielded significant alterations in the portal circulation, lasting for a prolonged period. Hepatic arterial vascular resistance showed a 2- to 4-fold increase during the first 8 hours following LPS administration (Figure 6). During this early septic phase, a significant portal hypertension was noticed, whereas measured PVP was elevated to approximately 225% of baseline values (Figure 7). Portal venous pressure showed a biphasic response after LPS administration. During the first 8 hours after LPS administration, PVBF decreased to approximately 51% of baseline (Figure 4). After a transit recovery to baseline, a hyperdynamic phase with an elevation of PVBF to 147% of baseline began at 30 hours (12 hours after LPS administration) and remained until the end of the study period (Figure 5). During this late septic period, HPVR was decreased to 63% of baseline and PVP was slightly increased to 121% of baseline (Figure 6 and Figure 7). DuP753 treatment significantly attenuated the effect of the second insult (LPS) on portal hemodynamics in burned animals. In contrast with untreated animals, HPVR in the treated group showed a transient increase of 14% at 2 hours after LPS administration, followed by a gradual decrease, reaching 41% of baseline 18 hours after LPS administration (Figure 6). DuP753 treatment ameliorated the LPS-induced portal ischemia/reperfusion insult in burned animals as PVBF showed after a moderate decrease of 15% at 2 hours after LPS administration, a steady increase with an average of 20% during the rest of the study period (Figure 5). Portal hypertension did not occur after LPS administration to burned animals treated with DuP753.

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**Figure 5.** Postburn blocking of angiotensin II receptors yielded a significant improvement in postlipsaccharide portal venous blood flow (PVBF, 85% of baseline vs 48% of baseline in nontreated animals, $P<.05$, analysis of variance). $P<.05$ for $*$ vs baseline, # vs sham, and for $¥$ vs treatment group (DuP753). LPS indicates lipopolysaccharide.

**Figure 6.** Hepatic portal vascular resistance (HPVR) in the 3 experimental groups. $P<.05$ for $*$ vs baseline, # vs sham, and for $¥$ vs treatment group (DuP753).

**Figure 7.** Portal venous pressure (PVP) was significantly elevated after burn and endotoxin, but not in the treatment group. $P<.05$ for $*$ vs baseline, # vs sham and for $¥$ vs treatment group (DuP753).
the post–LPS hyperdynamic phase, both DO2 and V·O2 were reman at baseline levels after LPS (Figure 8). During changed at this time point. Animals treated with DuP753 increased.

After an initial reduction, systemic DO2 and V·O2 showed a marked increase during the first 4 hours postburn (Figure 8). Administration of LPS yielded a significant drop in DO2 during the first hour. Systemic V·O2 was unchanged at this time point. Animals treated with DuP753 remained at baseline levels after LPS (Figure 8). During the post–LPS hyperdynamic phase, both DO2 and V·O2 were increased.

During the first 2 hours after the burn, hDO2 showed a significant drop to 53% of baseline, while hV·O2 decreased to 15% of baseline levels (Figure 9). In contrast, DuP753–treated animals did not show any marked alterations in hDO2 and hV·O2 after thermal injury (Figure 9).

The second insult (LPS) yielded a dramatic reduction in hDO2 to a level of 22% of baseline during the first hour after LPS administration and remained as low as 52% of baseline at 4 hours after LPS administration (Figure 9). Postburn treatment with DuP753 prevented this effect of LPS, as hDO2 showed a transient 16% increase at 1 hour after LPS and then remained at baseline level. hV·O2 showed a similar pattern, whereas a significant decrease of 30% to 63% of baseline values was calculated during the first 4 hours after LPS, followed by a 50% increase at 8 hours after LPS (Figure 9). Animals receiving DuP753 did not show any significant changes in their hV·O2 after LPS administration (Figure 9).

The renin-angiotensin axis seems to play an important role in the pathophysiology of thermal injury. Hilton et al. have reported a linear increase in plasma angiotensin II levels from 15 minutes to 6 hours after burn. Angiotensin II is a potent vasoconstrictor that exhibits important splanchnic selectivity, which is thought to be caused by an increased affinity of the angiotensin II receptors on the splanchnic vascular smooth muscle.

The selective improvement in hepatic arterial circula-
tion in the treatment group suggests that angiotensin II is one of the mediators, responsible for the postburn hepatic ischemia.

The second insult (LPS) resulted in a significant hepatic arterial vasoconstriction, with a 16-fold increase in HAVR, which was associated with a significant reduction of HABF during the first 4 hours after endotoxin (12% of baseline). During this early septic phase, HPVR showed a 4-fold increase with a subsequent 50% decrease in PVBF. Postburn administration of angiotensin II inhibitor DuP753 was found to ameliorate the effect of the second septic insult on both hepatic arterial and portal venous circulations. The noticed decrease in both HABF and PVBF after LPS administration cannot be the result of alterations in the systemic circulation, as postendotoxin CO and SVRI did not show any significant changes at these time points. The drop in PVBF could be explained as the result of the postinjury sepsis induced selective mesenteric ischemia, previously documented in the same model.6 The previously documented positive action of angiotensin II-receptor blocking on mesenteric blood flow26 could account for the observed improvement in post–LPS portal circulation after DuP753 treatment. However, the independent hepatic arterial vasoconstriction, seen after the second insult, together with the finding that treatment with angiotensin I-receptor blocker DuP753 ameliorated this process indicates that angiotensin II is one of the mediators, responsible for the adverse effect of postburn sepsis on hepatic arterial circulation. The adverse action of angiotensin II on postburn and sepsis hepatic arterial circulation seems to exhibit an important splanchnic selectivity, as a result of an increased affinity to angiotensin II receptors on splanchnic vascular smooth muscle.11 In humans, inhibition of angiotensin has been documented to decrease splanchnic vascular resistance under normotensive conditions.12

Lipopolysaccharide-induced reduction in hepatic blood flow could be argued to be not primarily caused by a direct action of angiotensin II, but secondary to the generation or inhibition of another mediator. Nitric oxide (NO) has recently been implicated in the pathophysiology of liver injury during ischemia/reperfusion and endotoxemia.13-16 In vitro study demonstrated that angiotensin II can decrease LPS-stimulated NO production, which is considered an endogenous nitrovasodilator,17 by inhibiting induction of inducible form of NO synthase expression.18 In another in vitro study, the effect of LPS on the angiotensin II receptor was found to be dose, time, and protein synthesis dependent and associated with an increased expression of the receptor gene 6.19 The ability of LPS to increase angiotensin II binding in cultured vascular smooth muscle cell was independent of the endotoxin induction of NO synthase. These results suggest that endotoxin may enhance the expression of cell surface receptors, which seems to be caused by nonspecific LPS-related induction of genes.

Another beneficial effect of angiotensin receptor blocking treatment was the prevention of the sepsis-induced portal hypertension. Postburn endotoxin-induced portal hypertension may account for the previously reported phenomenon of endotoxin-induced bacterial translocation.20 Acute portal hypertension has been previously shown to promote bacterial translocation.21 The underlying mechanisms are probably the disruption of the intestinal mucosal barrier (caused by acute venous congestion), increasing splanchnic blood pooling, edema and ischemia.22,23 Portal hypertension, initiated by endotoxin, has also been shown to induce hepatic microcirculatory disturbance, which may cause liver injury.24 Thus, postburn administration of DuP753 may decrease the incidence of hepatic injuries and bacterial translocation in the septic phase.

Early hypoxia in the splanchnic region is suggested as a plausible mechanism behind the development of secondary organ failure, especially in sepsis.25 In this study, hDO2 was significantly reduced during this early postburn phase. DuP753 treatment significantly improved hDO2 after thermal injury.

The second insult yielded a significant fall in hDO2. A pathologic flow-dependent hDO2 response was observed with a pronounced and prolonged hypoxic period. The development of flow-dependent liver hypoxia was shown before in a septic shock pig model and was reflected in a decrease in liver lactate turnover (increased liver lactate release) during late sepsis.26 Hepatic VO2 showed a pathologic hDO2 dependency, leading to oxygen debt that limits metabolism. This early decreased hVO2 indicates the inability of the liver to compensate adequate oxygen delivery by increasing oxygen extraction, resulting in tissue hypoxia. These results could be explained by a defect in microvascular regulation of blood flow that interfered with the optimal distribution of a limited DO2 in accord with tissue oxygen needs.26 Interaction with other mediators, such as NO, is also a possible pathway. Recently, NO has been shown to be involved in hepatic oxygen transport and consumption during endotoxemia.10,27 Our data demonstrate that these negative effects of LPS on hepatic oxygenation can be prevented by DuP753 treatment. Following LPS challenge, burned animals in the treatment group showed a significant improvement in their mesenteric oxygenation status, as compared with nontreated animals. The action of DuP753 seems to be selective. The enhancement in oxygen supply to meet the increased oxygen demand was only noticed in the hepatic circulation and no significant differences were found between treated and untreated animals, with respect to systemic DO2 and VO2.

**CONCLUSIONS**

The results of this study clearly show that angiotensin II plays a pivotal role in the process of hepatic ischemia and reperfusion injury induced by thermal trauma and endotoxemia. Postburn treatment with DuP753, a specific angiotensin II receptor antagonist, seems to ameliorate these adverse effects of burn and endotoxin on hepatic perfusion and oxygenation by enhancing hepatic blood flow and oxygen supply.

DuP753 was kindly provided by DuPont Merck Pharmaceutical Co, Wilmington, Del.
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REFERENCES