Trauma and Sepsis Induced Splanchnic and Hepatic Ischemia and Reperfusion Injury

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Trauma and Sepsis Induced Splanchnic and Hepatic Ischemia and Reperfusion Injury

Trauma en sepsis geïnduceerde intestinale en hepatische ischemie en reperfusie schade

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O WORLD, thou choosest not the better part
It is not wisdom to be only wise
And on the inward vision close the eyes
But it is wisdom to believe the heart
Columbus found a world, and had no chart
Save one that faith deciphered in the skies
To trust the soul's invincible surmise
Was all his science and his only art
Our knowledge is a torch of smoky pine
That lights the pathway but one step ahead
Across a void of mystery and dread
Bid, then, the tender light of faith to shine
By which alone the mortal heart is led
Unto the thinking of the thought divine

George Santayana (1863)

To

Jeroen and Chris

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CHAPTER 1

Introduction

Introduction

Multiple organ dysfunction syndrome (MODS) is widely considered to be the leading cause of morbidity and mortality in the surgical intensive care unit (ICU) [1-3]. Despite intensive investigation, the pathogenesis of MODS remains elusive. Literally hundreds of biochemical and cellular abnormalities have been described in patients with MODS, and their very number has made it difficult, if not impossible, to define a single common underlying event or process as the pathogenic basis of the disorder [4]. To date, several theories about the pathogenesis of MODS have been emerged. These theories have led to some conceptual models of MODS.

Pathophysiology

Infection and MODS

The first description of MODS emphasized its common association with occult or poorly controlled infection [5-7], frequently either peritonitis [8] or pneumonia [9]. However, more recent reports indicate that infection, although common in patients with MODS, is not necessarily present [10] and frequently follows, rather than precedes, the development of the syndrome [11, 12]. Microbial products such as endotoxin may also cause MODS. Endotoxemia is much more common in the critically ill patient than in documented infection [13] and correlates poorly with culture-proven infection [14], suggesting an important role for the absorption of endotoxin from the gastrointestinal tract [15].

Systemic Inflammation and MODS

Clinical evidence of systemic inflammation is manifest in almost all patients developing MODS [16, 17]. In fact, remote organ dysfunction can be considered the *functio laesa* of systemic inflammation [18]. Although it is difficult to differentiate the clinical manifestations of inflammation from the infections that may be their cause, it can be

demonstrated that the severity of the clinical inflammatory response, rather than the presence or absence of infection, is the more important determinant of ICU survival [19]. Although the synthesis and release of a panoply of biochemical mediators of inflammation is characteristic of both experimental and clinical sepsis, the mechanisms through which these molecules may induce organ injury are much less clear [20]. An alternate concept of the dysregulated inflammatory response that accompanies MODS suggests that the problem is not so much excessive inflammation as an acquired state of immunodeficiency or immune paralysis [21]. It seems realistic to consider that MODS reflects the state of dynamic imbalance that exists between each of the component mediators of the systemic inflammatory response.

Ischemia and Reperfusion Injury

Reduced oxygen delivery or utilization would be expected to inhibit the normal physiologic functions of the cell; therefore, the assumption that cellular hypoxia is the final common pathway to organ dysfunction is interesting [22]. Although adequate initial resuscitation usually restores oxygen delivery at the level of the whole organism, regional hypoxia in tissues such as the gastrointestinal tract [23, 24] is a well-documented phenomenon.

Reperfusion injury, precipitated by lack of oxygen, is likely to play a major role in the development of MODS. Certain tissues, such as the intestinal mucosa and hepatic parenchyma and nonparenchyma, may be especially susceptible because of the specific microvascular anatomy. Structural changes include not only swelling of the organelles but also the entire cell due to the entry of water and electrolytes. Lysosomal ruptures precede cell death. Oxygen free radical formation in the intestines and liver may trigger or cause injury in other distant organs, e.g. the heart and lungs, and affect overall vascular function [1, 25].

Increased circulating concentrations of lactate are also suggestive of tissue hypoxia, either global or regional, and are associated with an adverse outcome [26-28].

Apoptosis

Apoptosis describes a physiologic process through which cells activate an endogenous program that leads to the controlled death of the cell and its transformation to membrane-bound vesicles that are cleared by macrophages without evoking an inflammatory response [29]. The normal expression of apoptosis is fundamental to such processes as embryologic development, immune maturation, aging, and the resolution of inflammation. In critical illness, the expression of apoptosis appears to be altered. Apoptosis of lymphocytes and gut epithelial cells is increased [30, 31], whereas that of neutrophils is delayed [32-34].

Microvascular Coagulopathy

Multiple lines of evidence point to a pivotal role for inappropriate intravascular coagulation as a final common pathway to organ dysfunction. Cohort studies demonstrate a striking association between dysregulated coagulation and the development of organ dysfunction [35]. Alterations in levels of factors that regulate the balance between coagulation and fibrinolysis are common in critically ill patients and cause a shift toward a procoagulant state [4]. Changes in these parameters precede the development of organ dyfunction [36] and persist in those patients who develop organ dysfunction or die [37].

Gut-Liver Axis and MODS

The gut hypothesis of MODS has received considerable attention over the past several years [38, 39]. Intestinal mucosal injury can lead to dysfunction of the barrier between the intestinal lumen and blood, with translocation of intestinal bacteria and toxins to the circulation [40, 41]. Translocation is thought to play a substantial role in the pathogenesis of sepsis, systemic inflammatory response syndrome, and MODS, through stimulation of cytotoxic pathways [42]. Hence, the gut has been called the "primus motor" of MODS [43].

When translocated bacteria and endotoxin reach the liver, activation of Kupffer cells occurs, resulting in the secretion of proinflammatory mediators that enhance the stress response and alter various immune functions [44]. These mediators cause binding of neutrophils to endothelial cells, activate local and systemic coagulation mechanisms, and eventually cause direct end-organ injury. Therefore, it was proposed that the gut is the "starter" of MODS, whereas the liver is the "motor" of MODS [45].

Sequential Insults and Priming the Immune Response

There is accumulating evidence that various insults, such as uncontrolled infection, shock or trauma, can prime the organism such that a subsequent otherwise nonlethal bacterial or endotoxin challenge becomes lethal [1]. Experimental observations have been used to develop hypotheses that attempt to explain the exaggerated inflammatory response and organ dysfunction experienced by critically ill patients. These "two-hit" hypotheses imply that an initial sublethal insult establishes a physiologic state, which "primes" the organism to develop an amplified response to subsequent injuries [1, 45]. The resultant overwhelming inflammatory response is postulated to underline the pathogenesis of MODS in critically ill patients [46, 47].

Interventional Strategies and Therapeutic Options

Infection Control Measures

Despite the development of more powerful antibiotics, our ability to rescue patients from established MODS has not improved significantly since the syndrome was first described two decades ago [46]. Meta-analyses of infection control measures in clinical studies have revealed that the evidence that successful treatment of persistent or nosocomial ICU-acquired infections alters the outcome of MODS is far from compelling [48-51]. The pathogenic role of endotoxin is equally uncertain, as none of the few clinical trials that

have evaluated endotoxin neutralization as a therapeutic strategy have shown unequivocal evidence of benefit [52].

Immune Response Modulation

Clinical trials of a variety of strategies designed to inhibit inflammation in critical illness have generally yielded disappointing results [19]. The relatively modest impact of such approaches suggests that either the multiple redundant mediators must be targeted or that the ultimate morbidity of sepsis and MODS results from the downstream consequences of cytokine activity [18]. The lack of efficacy of early high-dose corticosteroids therapy [53, 54] or ibuprofen [55] strategies, that would be expected to reduce the levels of a number of inflammatory mediators, suggests that the latter interpretation may be more accurate [56].

Oxygen Delivery Enhancement

Despite advances in critical care, the development of better resuscitation and sophisticated ventilatory strategies, the mortality of multiple organ failure remains unchanged [57]. The initial enthusiasm for resuscitation to supranormal values to correct occult tissue hypoxia [58] has not been supported by more recent randomized trials [59]. As a matter of fact, the use of transfusion [60] or large doses of dobutamine [61] to increase oxygen delivery actually worsens outcome and increases the severity of organ dysfunction.

Apoptosis Regulation

Interventions that modulate the expression of apoptosis have been implicated in improving outcome in a variety of experimental models of inflammation [62, 63]. However, therapies targeting the expression of apoptosis are not yet clinically available. Therefore, the ability of such novel therapeutic strategies to prevent organ dysfunction in the critically ill patient remains unproven.

Coagulation Cascade Modulation

A variety of strategies that target the coagulation cascade have shown to improve survival in experimental models [64]. In human, however, the results of clinical trials are somehow conflicting. In human volunteers, infusion of tumor necrosis factor (TNF) activates coagulation and reproduces the coagulopathic profile of critical illness [65]; paradoxically, neutralization of TNF may also augment coagulation [66]. Early studies suggesting benefit of administration of antithrombin III [67, 68] were not replicated in a recent large phase III multi center trial [4]. The administration of recombinant activated protein C has been shown to improve outcome in a small cohort of patients [69]. Ongoing multicenter trials should provide the evidence of clinical efficacy and safety [69].

Prophylactic Measurements: Selective Gut Decontamination and Immunonutrition

Meta-analyses of the effects of infection prophylaxis using techniques of selective digestive tract decontamination show a striking reduction in rates of such infections as pneumonia, wound infection, and bacteremia, but a much more modest, albeit statistically significant, reduction in mortality [70, 71].

Selective gut decontamination showed no clinical benefits in multi-trauma patients. Early enteral nutrition, especially with immunomodulating ingredients "immunonutrition", decreases post-traumatic complications, but a reduction of mortality could not be described in severely injured patients so far [72]. Meta-analyses of the effect of immunonutrition in critically ill patients after trauma, sepsis, or major surgery, show significant reductions in infection rate, ventilator days, and hospital length of stay, but no effect on mortality [73]. Although it is tempting to conclude that early enteral nutrition reduces bacterial translocation by maintaining normal gut flora, enhancing mucosal integrity, and promoting local immune response, more appealing alternative explanations include that the gut is an immunologic organ that can be modulated to enhance systemic immune response or, through emphasis on use of early enteral nutrition, patients are not exposed early to the immunosuppressive effects of total parental nutrition [45].

Background and Objectives of the Thesis

MODS is, with an incidence of 10-25% and a mortality of 50-70%, the most severe complication after severe trauma [72]. MODS is a prototypical exemplar of the application of complexity theory to an understanding of the pathophysiology of critical illness [56, 74]. It arises through the interactions of a network of physiologic insults including injury, tissue ischemia, bacteremia, endotoxemia, the host inflammatory response, and the interventions used to sustain organ function during a time of otherwise lethal insufficiency. Its mediators are many and interdependent, with the activity of one inducing the expression of others that amplify, inhibit, or otherwise modify the expression of the process [19].

The implications of an understanding of the complex nature of organ dysfunction are critical to the development of rational strategies to prevent or treat the process. Strategies directed against events late in the process may be effective but are unlikely to have a significant effect on a process whose expression, at least from the perspective of the element targeted, has become autonomous [4, 18, 75]. In contrast, ischemia and reperfusion injury to both the intestine and the liver appears to be a relatively early event in the process of post-injury development of MODS, as outlined in figure 1. Therefore, a target-oriented approach including early augmentation of intestinal and hepatic perfusion and oxygenation seems conceptually a more attractive therapeutic option, either as a preventive measure for subjects at risk or as a promising treatment modality in the whole treatment strategy for patients with MODS.

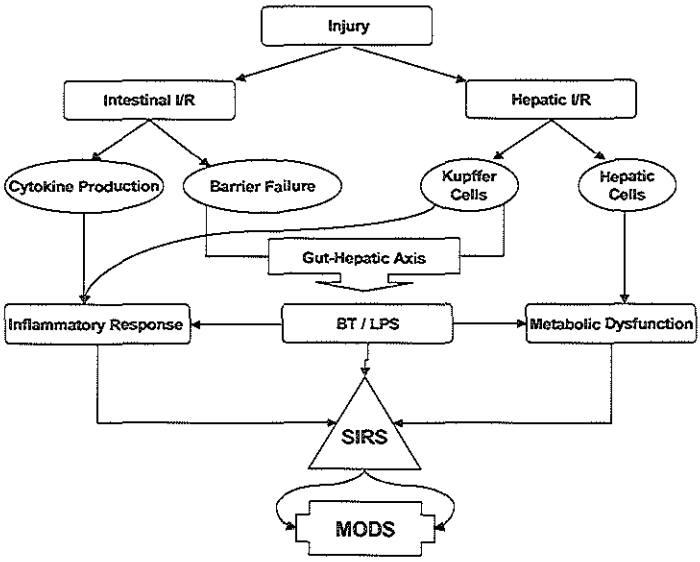

Fig. 1. Conceptual model of the role of ischemia and subsequent reperfusion injury (I/R) of the intestine and liver in initiating post-injury MODS.
BT (bacterial translocation), LPS (lipopolysaccharide), SIRS (systemic inflammatory response syndrome), MODS (multiple organ dysfunction syndrome).

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CHAPTER 2

Mediators and Intervention Modalities in Injury- and Sepsis-Induced Intestinal Ischemia

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In Faist E BA, Schildberg FW, eds., ed. The Immune Consequences of Trauma, Shock and Sepsis Mechanisms and Therapeutic Approaches, Vol. 1. Legerich, Germany: Pabst Science Publishers, 1996. pp. 153-162.

Introduction

Occurrence of intestinal ischemia after trauma has recently been recognized as a frequent and significant phenomenon. A clinical review of the autopsy records of severely burned patients revealed a 61% incidence of intestinal ischemia [14]. At the time of death, sepsis was manifested in more than 80% of these patients.

The relationship between gut ischemia and sepsis remains to be defined. Splanchnic ischemia has been reported to alter the intestinal mucosal barrier, leading to the escape of indigenous bacteria and their endotoxins to the systemic circulation and remote body organs [20,23,34], a process termed "bacterial translocation" [4]. There is accumulating evidence from experimental studies indicating that bacterial translocation and endotoxin release have a potential role in the pathogenesis of sepsis and multiple organ failure [9,34]. Clinical evidence of disruption of the intestinal barrier is increasing. Infections with gut-associated bacteria with no detectable pathologic focus have been documented in victims of trauma [5]. It has been postulated that intestinal ischemia and sepsis can become self-sustaining (Fig. 1), since endotoxin, either gut- or tissue-derived, has been documented to decrease intestinal blood flow and promote bacterial translocation [12,26,36].

Several factors can contribute to the development of post-trauma or sepsis-induced intestinal ischemia, including systemic hemodynamic status, condition of the microcirculation, circulating vasoactive substances, humoral and cellular mediators, and the products of local cellular metabolism.

Systemic hypovolemia and cardiac failure may result in splanchnic ischemia [1,16]. The measured reduction in the mesenteric blood flow does not correlate with cardiac output. Post-injury splanchnic ischemia occurs in the absence of depression of cardiac output or systemic blood flow [23,33], indicating that the mesenteric blood flow can be independently regulated by certain mediators.

The identification of these mediators will facilitate the development of interventions to modulate the mesenteric blood flow and prevent the manifestation of subsequent pathological events.

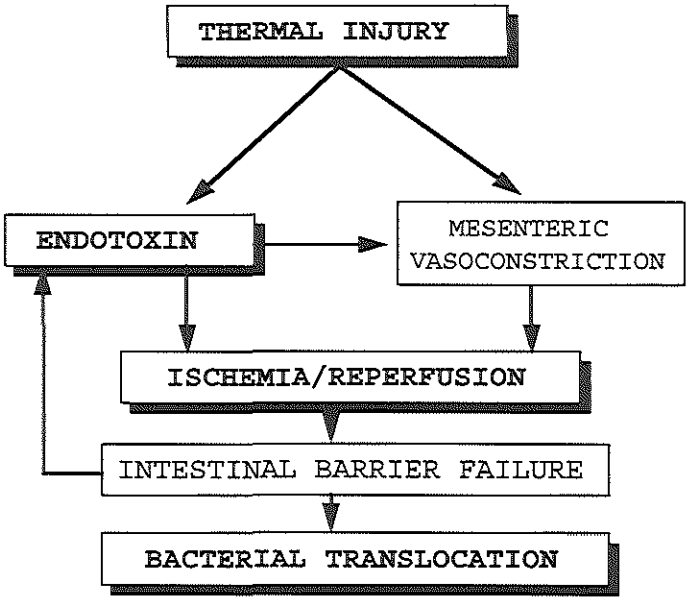

Fig. 1: Thermal-induced intestinal ischemia and reperfusion injury can initiate bacterial translocation and systemic endotoxemia. Endotoxin, either gut- or tissue-derived, acting synergistically with the first insult may create a self-sustaining septic state.

Mediators of the Post-Trauma and Sepsis-Induced Intestinal Ischemia

It has been recognized that the severity of ischemia-induced intestinal injury is inversely related to mesenteric blood flow. Recently, it has become apparent that mesenteric vasculature reacts in a very sensitive way to injury. In an ovine model, a 40% body surface area (BSA) burn was demonstrated to decrease blood flow through the cephalic mesenteric artery by 50% during the first hour postburn [24]. In a porcine model, a 40% BSA burn reduced the blood flow of the superior mesenteric artery (SMA) to 25% and 30% of baseline 2 and 4 hours after burn, respectively [33]. The impact of endotoxemia on mesenteric blood flow has been investigated. Endotoxin has also been

reported to selectively alter intestinal blood flow and cause a profound mesenteric vasoconstriction. In a rat model, the administration of endotoxin yielded a 25% and 50% decrease in blood flow to the distal ileum and cecum, respectively [36].

The systemic hemodynamic changes occurring during the early phase of both post-injury and septic shock have been postulated to alter the splanchnic circulation. Schlag and associates have demonstrated gastrointestinal ischemia in a hypovolemic-traumatic baboon model [27]. Although hypovolemia, cardiac failure and increased systemic vascular resistance may contribute to this phenomenon, the pathway by which they interfere with mesenteric circulation seems to be nonspecific. The data of several experiments using different models demonstrate that the decrease in intestinal blood flow does not correlate proportionally with systemic hemodynamic failure and that thermal injuries and endotoxins can reduce the intestinal blood flow in hyperdynamic states or in situations with normal indices of perfusion, i.e. cardiac output, blood pressure, and urine output [24,33,36]. Thus, the mesenteric blood flow must have been altered in these situations by certain mediators which express specific mesenteric vasoconstrictive capacities.

The systemic inflammatory response to severe injuries and/or sepsis encompasses several cellular interactions, leading to the generation of diverse proinflammatory cytokines and vasoactive peptides. The vasoactive metabolite of arachidonic acid, thromboxane A_2 (TxA_2), appears to play a pivotal role in the process of mesenteric ischemia following burn or sepsis. TxA_2 has been shown to produce vasoconstriction of many vessels, including the celiac and mesenteric arteries. Herndon and his group have documented a marked elevation of tromboxane (Tx) B_2, the stable metabolite of the potent vasoconstrictor TxA_2, in the serum of patients immediately after burn and during septic episodes [19]. TxA_2 has also been implicated in the pathophysiology of endotoxic shock [30]. An intravenous bolus of endotoxin elicits a marked but transient increase in plasma TxB_2 and 6-keto-PGF1α in a large number of species. A smaller, delayed and more prolonged increase in TxB_2 and 6-keto-PGF1α is reported in animals with septic shock, i.e. those with fecal peritonitis or cecal ligation [2]. Using a burn/endotoxin ovine model, burn tissue endotoxin has been shown to stimulate local TxA_2 production leading

to distant lung dysfunction without the need for circulating endotoxin. This study has clearly demonstrated that the source of the TxA_2 is the burn, while with endotoxemia the source is the lung [13].

Other arachidonic acid metabolites, such as leukotriene (LT) C4 and D4, may also be involved in the process of mesenteric vasoconstriction.
These are potent smooth muscle vasoconstrictors. Both compounds have been found in high concentrations in severely injured patients [37]. Cohn et al. [8] examined the effects of the intravenous infusion of graded doses of LTC4 on the mesenteric blood flow in immature swine. Infusing LTC4 significantly decreased the mesenteric blood flow and mesenteric oxygen uptake and significantly increased ileal intramucosal hydrogen ion concentration.

Recently, two vasoconstrictor peptides have been shown to increase after trauma and selectively affect the splanchnic vasculature. In an anesthetized dog, vasopressin and angiotensin II levels were measured by radioimmunoassay. Vasopressin plasma levels were increased 4- to 6-fold 15 minutes postburn and remained elevated for 6 hours. Angiotensin II increased 4-fold in a linear manner between 15 minutes and 6 hours post-injury [21]. In a canine model of occlusive mesenteric ischemia, the preferential constriction of the ischemic segments of intestine by vasopressin was demonstrated. Similarly, a differential vasoconstriction is observed in the response of the splanchnic circulation to angiotensin II. This phenomenon is likely related to the relatively great number of angiotensin II receptors in the splanchnic vascular smooth muscle [17]. In an experimental model of nonocclusive mesenteric ischemia, disproportionate ischemia was seen secondary to splanchnic vasospasm [22]. These hemodynamic changes correlated closely with plasma renin levels and could be reproduced, in the absence of shock, by the infusion of angiotensin II directly into the mesenteric vessels. In addition, the histological intestinal damage resembled that seen with nonocclusive mesenteric ischemia.

Mesenteric Ischemia and Bacterial Translocation

The intact intestinal mucosa acts a mechanical and immunological barrier to indigenous bacteria and antigens. Factors which adversely interfere with this defense mechanism may lead to an increased translocation of viable bacteria or endotoxins from

the confines of the intestine to other body systems. Recent studies have clearly documented that the barrier function of the intestinal mucosa is deranged in experimental animals subjected to mesenteric ischemia [3]. In an ovine model, just mechanical reduction of the blood flow with the use of pneumatic occluders caused bacterial translocation [23]. Intestinal ischemia induces a spectrum of injury from relatively subtle changes in mucosal capillary permeability to gross transmural infarction, depending on severity and duration. Both burn and endotoxin have been shown to induce a profound mesenteric ischemia, mucosal damage and bacterial translocation. In a mouse model, grossly ulcerated ileal and cecal mucosa was found within 24 hours after 25% BSA burn [11]. Endotoxin has been reported to damage the intestinal mucosal barrier and consequently promote the translocation of various indigenous bacteria [10]. Histological examination of the intestine after endotoxin administration revealed edema of the ileal and cecal lamina propria with separation of the epithelium from the villus tip. Although the exact mechanisms responsible for the intestinal injury have not yet been described, it appears that there are basically two events that can induce intestinal tissue injury in ischemic states, namely, hypoxia during the ischemic period and generation of oxygen free radicals following ischemia at reperfusion [18]. Granger and associates have extensively studied the pathophysiology of ischemia/reperfusion intestinal injury [38]. The results of these studies suggest that superoxide radicals are involved in the pathogenesis of ischemic mucosal injury and that the enzyme xanthine oxidase is the source of superoxide radicals in the ischemic small bowel.

Interventions to Prevent Intestinal Ischemia Following Injury and Sepsis

The essential role of adequate resuscitation and maintenance of fluid balance in the care of major trauma and sepsis have been long established. Correction of hypovolemia and improvement of cardiac output can certainly be beneficial to mesenteric circulation and intestinal oxygenation. In a recent study, the effects of hypertonic versus isotonic crystalloid resuscitation on intestinal microcirculation were demonstrated in a porcine hemorrhagic shock model [15]. Resuscitation with hypertonic saline solutions restored the intestinal mucosal blood flow, as measured by a laser Doppler flow probe, to baseline levels. In contrast, isotonic fluid resuscitation failed to restore splanchnic

perfusion. In a porcine burn model, a 33% decrease in mesenteric blood flow was demonstrated in animals resuscitated with only Ringer's lactate (RL) solution. This effect could be blocked by a prior administration of hypertonic saline/dextran solution (HSD) [32]. The HSD solution prevented the early increase in mesenteric resistance and the decrease in mesenteric O_2 consumption seen after burns when RL alone was used for resuscitation. These results indicate that hypertonic resuscitation may be beneficial in improving the post-injury microcirculation.

In an ovine model, the effects of nonspecific vasodilators on the mesenteric circulation were demonstrated (Fig. 2) [25]. The mesenteric blood flow was reduced after 40% BSA burn by 40% within the first 6 hours. Selective infusion of nitroprusside into the mesenteric artery after the burn prevented the fall in the mesenteric blood flow. In the same study, this treatment modality decreased the incidence of bacterial translocation to systemic organs from 88% to 24%. The administration of nonselective vasodilators maintains the splanchnic circulation after thermal injury. However, the nonspecificity of various vasodilators with their undesirable systemic effects makes it important to identify selective pharmacological agents.

Thromboxane appears to be one of the primary mediators in the process of burn and sepsis-associated intestinal ischemia. The impact of inhibition of thromboxane synthesis has been investigated. The effect of OKY-046, a specific thromboxane synthetase inhibitor, was investigated in a 40% BSA burn porcine model. The drug was given as a bolus of 10 mg/kg prior to burn and then as a continuous infusion at 10 mg/kg/min. OKY-046 was found to prevent the significant increase in the mesenteric vascular resistance seen during the first 8 hours after burn. The mesenteric blood flow was reduced to 25% and 30% of baseline at 2 and 4 hours after burn, respectively. This significant postburn decrease in mesenteric blood flow was not observed in burned animals receiving OKY-046. In the same study, the incidence of postburn bacterial translocation was significantly decreased (31% to 7%) after the administration of the thromboxane synthetase blocker OKY-046 [31].

In an endotoxic shock rat model, thromboxane synthetase inhibition was documented to improve the splanchnic circulation previously reduced by endotoxin [29]. The effects of two structurally dissimilar inhibitors of thromboxane synthetase were

documented. An imidazole derivative, 7-IHA, and OKY-1581 were injected intravenously at 30 and 5 mg/kg, respectively, prior to endotoxin. Both thromboxane synthetase inhibitors significantly improved the splanchnic blood flow, which was 55% reduced after endotoxin.

In a porcine endotoxic shock model, pretreatment with LY171883, a specific (LT) D4/E4 receptor antagonist, was evaluated. Normal mesenteric perfusion and oxygen delivery (DO_2) were maintained in the animals receiving LY171883, whereas in controls these parameters decreased significantly [7]. In another study, the administration of LY203647, a specific LT receptor antagonist, was reported to prevent the LTC4-induced mesenteric hypoperfusion and intestinal intramural acidosis [8].

Recently, we have completed a study to determine the efficacy of blocking the action of angiotensin II in sepsis and thermal injuries [28]. In an attempt to mimic the clinical situation, which is commonly complicated by sepsis and endotoxin release [35], we utilized a combined burn and endotoxin porcine model. Burn caused a significant increase (198% vs. baseline) of the mesenteric vascular resistance. Postburn endotoxemia significantly reduced the blood flow in the superior mesenteric artery to 60% of baseline. Treatment with DuP753, a specific angiotensin II receptor antagonist, prevented the postburn vasoconstriction and subsequently abrogated the impact of the postburn endotoxemia on the superior mesenteric artery blood flow. The incidence of burn- and sepsis-induced bacterial translocation was markedly reduced by Dup753 from 86% to 29%. This study indicates that the angiotensin II receptor inhibitor DuP753 has the potential to prevent trauma- and sepsis-induced mesenteric hypoperfusion and consequently attenuate the occurrence of bacterial translocation.

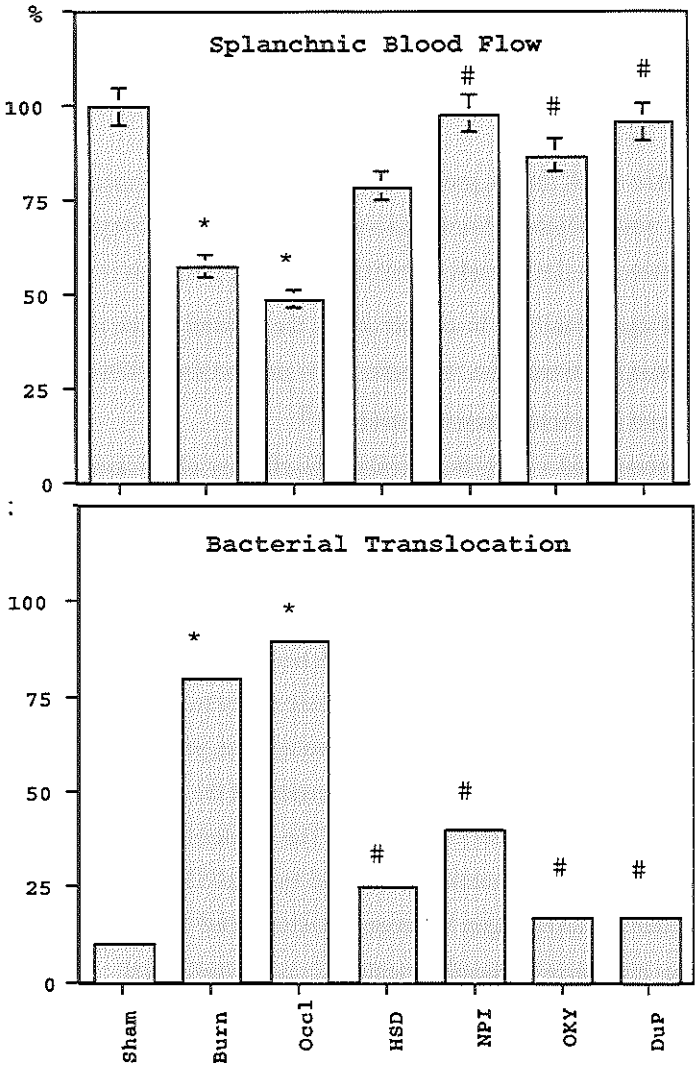

Fig. 2: The impact of 40% third-degree burn and mechanical occlusion (occl) on mesenteric blood flow and associated incidence of bacterial translocation. A summary of nonspecific, hypertonic saline dextran resuscitaton (HSD) [32] and nitroprusside (NPI) (unpublished data), and specific thromboxane synthetase inhibitor OKY-046 [31] and angiotensin II receptor antagonist DuP753 (DuP) [28], interventions and their effects in a 40% BSA, third-degree burn, porcine model. * $P<0.05$ vs. sham; # $P<0.05$ vs. burn.

Summary

Major trauma, such as severe thermal injury, appears to have a profound impact on intestinal blood flow. The subsequent post-trauma intestinal ischemia has been implicated as the cause of translocation of indigenous bacteria and endotoxin to remote body systems, which in turn may potentiate the development of sepsis and multiple organ failure. Sepsis, gut-derived or from other origins, with circulating endotoxin also appears to adversely interact with mesenteric circulation. The subsequent sepsis-induced mesenteric ischemia acts synergistically with the initial injury, promoting mucosal damage and bacterial translocation. Although different mechanisms may be involved, the generation of vasoactive substances and proinflammatory mediators seems to play a pivotal role in these processes. It is of great importance to identify the mediators which initiate ischemic insult and establish specific preventive modalities. So far, two vasomediators appear to be primarily responsible for these complications, TxA_2 and angiotensin II. Blocking the actions of these mediators by specific antagonists has been shown to ameliorate the post-injury and sepsis mesenteric vasoconstriction and attenuate bacterial translocation (Fig. 3).

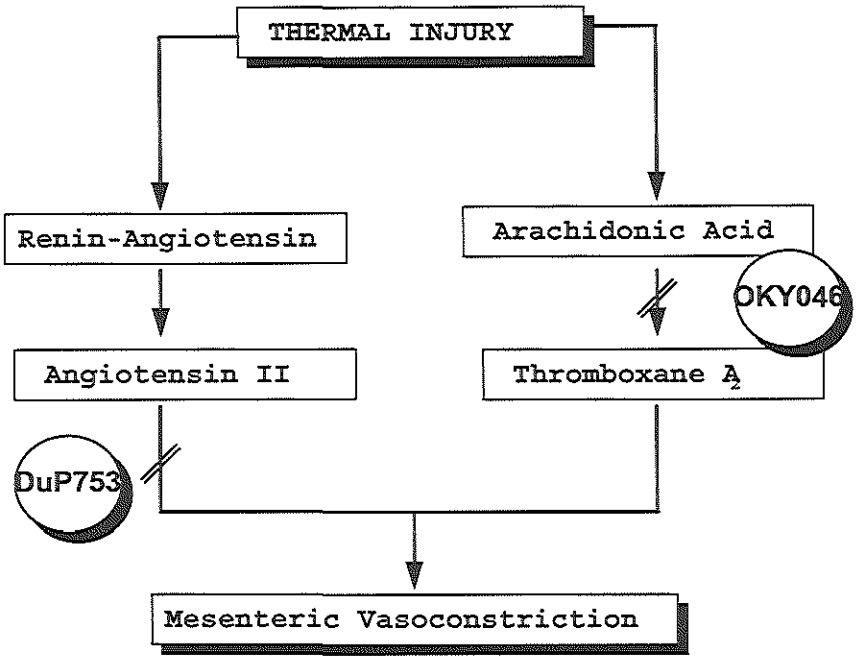

Fig. 3: Thromboxane A_2 and angiotensin II appear to be the primary mediators of postburn mesenteric vasoconstriction. By blockade of these pathways with specific inhibitors (OKY-046 and DuP753), postburn mesenteric ischemia is prevented and subsequent bacterial translocation is attenuated.

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CHAPTER 3

Burn- and Endotoxin-Induced Bacterial Translocation: Role of Hepatic Ischemia and Reperfusion Injury

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In Faist E BA, Schildberg FW, eds., ed. The Immune Consequences of Trauma, Shock and Sepsis Mechanisms and Therapeutic Approaches, Vol. 1. Legerich, Germany: Pabst Science Publishers, 1996. pp. 966-972.

Despite continuous research efforts, increasing knowledge and experience, advanced treatment modalities and sophisticated medical techniques, sepsis and multiple organ failure (MOF) remain the most challenging clinical syndromes of this century. While sepsis and MOF are responsible for 50% - 80% of all surgical intensive care unit deaths, the complex pathophysiology of these syndromes remains to be fully elucidated [1]. In recent years, the gastrointestinal tract has been implicated in the pathogenesis of these life-threatening syndromes [10]. It has been postulated that enteric organisms or their toxins escape the intestinal mucosal and act as triggers to initiate or exacerbate the septic status and thereby promote the development of sepsis [3]. The clinical significance of bacterial translocation is controversial. Whether it is solely a pathological process, the occurrence of which may initiate sepsis and MOF, or a biological event commonly found in animals, is still a subject of debate. At the present time, there is a body of evidence that bacterial translocation seen in different animal models is generated by an unbalanced ratio between gut bacterial growth, intestinal mucosal barrier and host immune response [5]. Thermally injured patients have alterations of each during the course of their illness.

The association between thermal injuries and dysfunction of the intestinal mucosal barrier has been clearly documented. A significant increase in permeability of rat small intestine was documented as early as 6 hours following 20% body surface area (BSA) scald burn [2]. Similar changes in intestinal permeability were found in burn patients during the first 24 hours after injury [4]. Using the lactulose-mannitol clearance assay, a 3-fold increase in intestinal permeability to lactulose was demonstrated in infected thermally injured patients [17]. This alteration in the mucosal barrier may contribute to the translocation of indigenous bacteria and endotoxins into the portal circulation [3]. As the hepatic mononuclear phagocytic system (Kupffer cells) appears to play a pivotal role in the clearance of translocating bacteria and/or endotoxin from the portal circulation, 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.

Phagocytosis of bacteria and sequestering of endotoxin by hepatic Kupffer cells impose a high metabolic demand of the mononuclear phagocytic system. Intermediary

metabolism and energy production have an absolute dependence on oxygen. Since oxygen cannot be stored intracellulary, inadequate oxygen availability rapidly leads to cellular dysfunction and ultimately cell death, with the net result being organ failure, hence, the importance of the hepatic perfusion for the performance of Kupffer cells.

Burn injuries have been documented to have a negative impact on hepatic perfusion. The influence of severe thermal injury on hepatic blood flow was investigated in a 50% BSA burn rat model, using the tricarbocyanine dye indocyanine green (ICG) [11]. Hepatic blood flow was decreased significantly by 0.5 hour postburn and remained approximately 20% below normal for 24 hours. The intrinsic efficiency of the liver in removing the ICG dye from the systemic circulation was also reduced.

In an ovine model the effect of a 40% BSA third-degree burn on effective liver blood flow was determined, using the galactose infusion technique [7]. The effective liver blood flow was decreased by 50% in the first 5 hours after burn, even when the animals were resuscitated to baseline cardiac output values.

The second insult, which may influence the gut-hepatic axis, is the release of endotoxins into the circulation. Burn patients are often subjected to episodes of transient endotoxin showers, which might be gut-derived or from other sources, such as the burn wound and the frequent surgical procedures [6,15].

The effect of endotoxemia on the hepatic circulation was evaluated in chronically instrumented and sedated sheep receiving a continuous intravenous infusion of *Escherichia coli* endotoxin [12]. The response of the hepatic artery was biphasic and consisted of a transient vasoconstriction followed by a transitory increase in hepatic artery blood flow, reaching a maximum of 921% of baseline values after approximately 2 hours.

Since thermal injury inducing hepatic ischemia may prime the host for an exaggerated response to subsequent insults such as bacteria or endotoxin, we investigated the impact of postburn endotoxemia on the gut-hepatic axis.

Female mini-pigs were chronically instrumented with transit time ultrasonic flow probes and catheters in the superior mesenteric and left hepatic veins. Arterial and Swan-Ganz catheters were also inserted. Six animals received a 40% BSA, third-degree flame burn and were resuscitated according to the Parkland formula (4 mL/kg/%BSA burn).

Eighteen hours after burn, 100 μg/kg *E. coli* lipopolysaccharide (LPS) (0111:B4; Difco, Detroit, MI) was intravenously administered.

Despite a moderate increase in the cardiac output, the blood flow in the common hepatic artery showed a transient but significant decrease (39%) shortly after burn. The second insult (LPS) yielded a dramatic and prolonged reduction of hepatic arterial blood flow during the first 4 hours after endotoxin (22%-77% of baseline, $P<0.05$). After recovery to baseline, a reperfusion episode followed, with an elevation of the hepatic artery blood flow to 152% of baseline values ($P<0.05$) at 8 hours post-endotoxin (Fig. 1).

The pronounced ischemic effect of endotoxin on the hepatic arterial blood flow, observed in this study, which is not in agreement with the previous report, might be due to the fact that different species and administration schemes were used. However, it is more likely that the priming impact of the early burn injury was responsible for the amplification of tissue response to endotoxin.

Under physiologic conditions, the regulation of hepatic arterial blood flow tends to buffer the impact of portal venous blood flow changes on total hepatic blood flow in order to maintain the latter constant. The function of portal venous blood flow as the major intrinsic regulator of hepatic arterial tone is known as the hepatic arterial buffer response [8]. This buffer function appears to depend on portal blood flow washing away local concentrations of adenosine from the area of the arterial resistance site [9]. Thus, a reduction in portal blood flow causes an increase in local adenosine levels, resulting in arterial dilation. Since the portal venous blood flow is determined by the vascular resistance of the intestine and, to a lesser extent, the spleen and pancreas, a decrease in the mesenteric blood flow would result in a compensatory increase in hepatic blood flow to maintain liver function.

In contrast with this scenario, in our model of postburn endotoxemia, the response of the hepatic arterial blood flow was unrelated to changes in portal circulation. The early postburn transient hepatic vasoconstriction occurred prior to changes in both portal and mesenteric circulations, demonstrating the relative independence of hepatic arterial response in relation to other splanchnic blood flow. This ischemic insult may explain the occurrence of transient hepatic function disorders, commonly seen early postburn, and may also adversely influence the phagocytic capacities of hepatic macrophages. It is also

possible that this hepatic ischemic insult may prime hepatic macrophages, leading to release of certain vasomediators, such as thromboxanes, which have been implicated in the pathophysiology of the postburn mesenteric vasoconstriction [14].

Despite the marked reduction in portal venous blood flow during the first 6 hours after endotoxin, the hepatic arterial blood flow showed a significant decrease for a period of 4 hours indicating a loss of the hepatic arterial buffer response (Fig. 1).

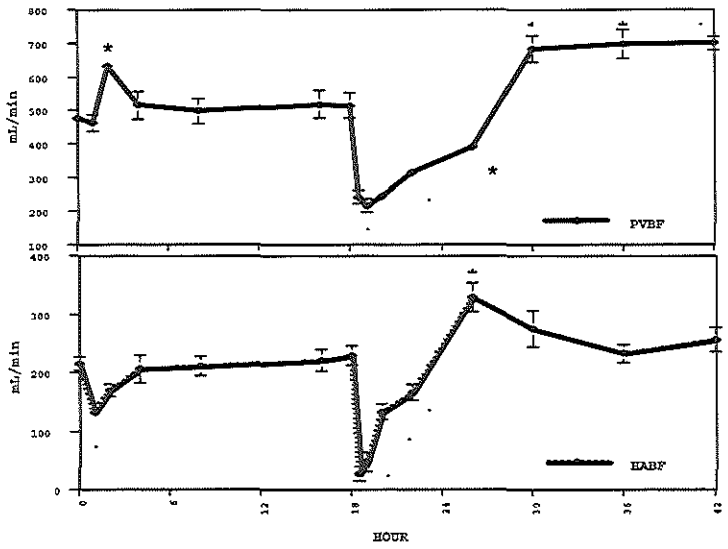

Fig. 1: Impact of burn injury, followed by a second insult (endotoxin) on hepatic arterial blood flow (HABF) and portal venous blood flow (PVBF). The loss of the hepatic arterial buffer response is demonstrated. Data are presented as mean ± SEM; *P<0.05 vs. baseline, Dunnett's test.

Although the precise mechanisms underlying this phenomenon remain to be identified, there are indications that hepatic Kupffer cells play a central role in this process. It has been established that the major controls of hepatic arterial and portal venous vascular resistance are presinusoidal [8]. It is also known that mixing between the two inflows occurs at the entrance of the sinusoids. The actual connections between arterial and portal

blood flow occur presinusoidally both by direct arterioportal anastomoses and through the peribiliary capillary beds. Thus, potential mediators released by hepatic parenchymal cells are not able to reach the area of vasculature that regulate blood flow. The early postburn increase in hepatic vascular resistance, together with the observed pronounced vascular resistance in both hepatic arterial and portal venous circulations, implicates presinusoidal Kupffer cells in this process. The magnitude of the hepatic response following the second insult may demonstrate the priming effect of the first insult.

The observed postburn reduction of the portal vascular resistance associated with increased portal venous blood flow together with the previously documented damage of the mucosal barrier caused by the mesenteric ischemia and reperfusion injury [4] are likely to be responsible for the washout of the indigenous bacteria or endotoxin to the liver. However, the induced hepatic ischemia and reperfusion injury might be the determining factor in the systemic spread of these bacteria or endotoxin. The profound action of endotoxin on the hepatic circulation, resulting in a long period of ischemia followed by reperfusion, may impair the phagocytic and bacterial clearance capacities of the hepatic macrophages, leading to spillover of indigenous bacteria and endotoxin to the systemic circulation. In the present study, postburn endotoxemia clearly promoted bacterial translocation (83%). The marked portal hypertension, seen in the postburn endotoxemic period (Fig. 2), may account for the occurrence of bacterial translocation and contribute to the previously reported phenomenon of endotoxin-induced bacterial translocation [16]. Acute portal hypertension has been previously shown to promote bacterial translocation [13]. The underlying mechanisms are probably the disruption of the intestinal mucosal barrier, caused by acute venous congestion, edema and ischemia.

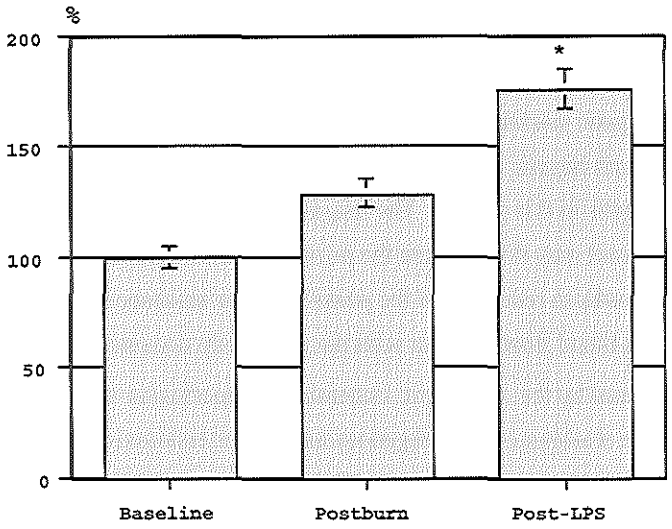

Fig. 2: Changes in the superior mesenteric venous pressure after burn and endotoxin. Data are given as a percentage of baseline; *P<0.05 vs. baseline, Dunnett's test.

In conclusion, thermal injury appears to induce hepatic ischemia, which may alter the hepatic bacterial clearance function, resulting in systemic bacterial or endotoxin spillover. The magnitude of the hepatic response to a second insult (endotoxemia) is magnified and manifested as a pronounced hepatic ischemia and reperfusion injury. Primed hepatic Kupffer cells are possibly accountable for this response. The associated increased portal venous resistance and portal hypertension may contribute to the occurrence of intestinal mucosal damage and subsequent bacterial or endotoxin translocation (Fig. 3).

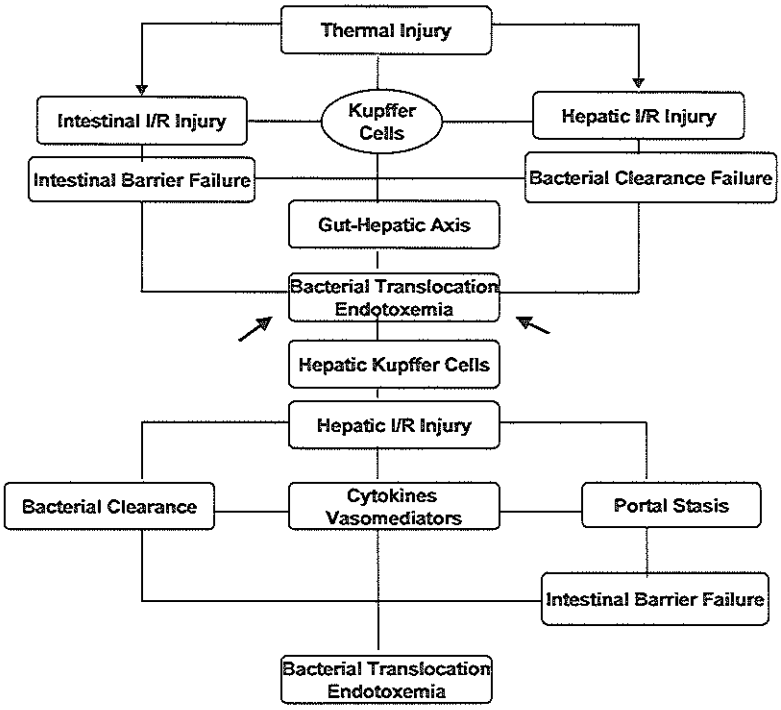

Fig. 3: Proposed mechanisms by which thermal injury interacts with the gut-hepatic axis, resulting in bacterial translocation and endotoxemia. The possible interplay between the profound impact of a second insult (bacterial translocation or endotoxemia) and the amplified hepatic response is also delineated.

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CHAPTER 4

Burn- and Endotoxin-Induced Gut Ischemia and Hypoxia
Role of Angiotensin II

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Abstract

Objective: To investigate the role of angiotensin II as a mediator of burn- and sepsis-induced gut ischemia and reperfusion injury, and the possible beneficial effects of angiotensin II inhibitor DuP753.

Summary Background Data: A second insult (endotoxemia) following burn may amplify intestinal ischemia and reperfusion injury. Angiotensin II, the production of which has been reported to increase after burn, is thought to be one of the primary mediators of postburn mesenteric vasoconstriction.

Methods: Eighteen female pigs were instrumented with an ultrasonic flow probe on the superior mesenteric artery and a catheter into the superior mesenteric vein. After 5 days, all animals were anesthetized, and 12 received 40% total body surface area third-degree burn. Eighteen hours after burn, 100 μg/kg *Escherichia coli* lipopolysaccharide (LPS) was intravenously administered. DuP753 was administered intravenously at 1 μg/kg to 6 pigs immediately after burn. The animals were studied for 42 hours.

Results: Burn caused a significant decrease in mesenteric arterial blood flow (Qm), to approximately 58% of baseline. Postburn endotoxemia significantly reduced Qm to 53% of baseline. Treatment with DuP753 prevented postburn vasoconstriction and subsequently abrogated the impact of postburn endotoxemia on Qm (postburn: 386±21 vs. 207±11 mL/min*, post-LPS: 372±23 vs. 202±18 mL/min*, *= $P<0.05$, Bonferroni t test). Mesenteric oxygen supply (mDO_2) was significantly reduced after burn and endotoxin to 60% and 51% of baseline, respectively. DuP753 administration significantly improved mDO_2 after both insults (postburn: 49±14 vs. 23±03 mL/min*, post-LPS: 40±08 vs. 19±37 mL/min*, *= $P<0.05$, Bonferroni t test). Burn- and LPS-induced mesenteric hypoxia, as indicated by decreased mesenteric oxygen consumption (mVO_2), was also ameliorated by DuP753 treatment.

Conclusions: These results indicate that angiotensin II is one of the mediators of the burn- and sepsis-induced gut ischemia. Angiotensin II inhibitor DuP753 can attenuate these events.

Introduction

Despite recent advances in the care of thermally injured patients, sepsis remains the most common cause of the high morbidity and mortality rates following major burn injuries.[1] Although the precise mechanisms have not yet been elucidated, the gastrointestinal tract has been postulated to be a major contributor to postburn sepsis.[2] The intestinal mucosal barrier normally prevents the enteric bacteria and their products from escaping and reaching extraintestinal organs. However, under certain circumstances the integrity of intestinal mucosa appears to be disrupted, allowing spread of endogenous bacteria or endotoxins; a process called bacterial translocation.[3,4] Previous experimental studies have shown bacterial translocation to occur in a number of conditions including burn and endotoxemia.[5,6] Burn injury has been demonstrated to induce intestinal mucosal damage and bacterial translocation. This mucosal injury appears to be due to mesenteric ischemia.[7] Endotoxin has been shown to increase intestinal permeability and promote bacterial translocation.[8,9] There is evidence that intestinal ischemia and reperfusion injury underlay this particular action of endotoxin.[10,11] Patients with major burn injuries might be particularly susceptible to factors associated with bacterial translocation, since they appear to be frequently subjected to repeated episodes of endotoxemia.[12] In turn, endotoxin has been reported to act synergistically to promote mucosal damage and bacterial translocation after major burn injuries.[13]

The molecular mechanisms underlying the observed intestinal ischemia in such situations are not yet known. Mesenteric vasoconstriction may be the result of a plethora of vasomediators, one of which is angiotensin II. The production of angiotensin II has been reported to increase significantly after thermal injuries.[14] Therefore, we postulated that angiotensin II may be one of the primary mediators of postburn mesenteric vasoconstriction. In order to evaluate the role of angiotensin II in this response, we investigated in this study whether the administration of DuP753, an angiotensin II antagonist, would attenuate burn- and endotoxemia-induced gut ischemia and reperfusion injury in a chronic porcine burn/sepsis model

Materials and Methods

The following experimental protocols were approved by the Animal Care and Use Committee of the University of Texas Medical Branch (ACUC #90-09-103).

Eighteen female mini-pigs (weight 20-25 kg) were prepared surgically 5 days before the experiment. After an overnight fast, the pigs were sedated with intramuscular ketamine (10 mg/kg) and mechanically ventilated with 2% to 2.5% halothane after endotracheal intubation. A bilateral subcostal incision was performed. A transit time ultrasonic flow probe (6-8 mm, Transonic Systems Inc., Ithaca, NY) was placed on the superior mesenteric artery. A 6.5F catheter was positioned in the superior mesenteric vein. A Witzel jejunostomy was also performed using a 12F Foley catheter. The abdomen was closed in layers.

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. Through a neck incision, a catheter was placed via the right common carotid artery into the abdominal aorta and a Swan-Ganz thermal dilution catheter (Model 93 A-131-5F, American Edwards Laboratories, Anasco, PR) was positioned in the pulmonary artery via the right jugular vein. A 12F Foley catheter was inserted in the urinary bladder.

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

The pigs were randomized into 3 groups:

1) The burn/lipopolysaccharide (LPS) group (n=6) had a 40% total body surface area (TBSA) third-degree flame burn under general anesthesia, as described above. The pigs were resuscitated according to the Parkland formula and received lactated Ringer's solution (4 mL/kg/% TBSA burned) starting immediately after the burn; half was given in the first 8 hours after burn and the remainder in the next 16 hours. Eighteen hours after burn, 100 μg/kg *E. coli* LPS (0111:B4; Difco, Detroit, MI) was administered intravenously. During the second day of the experiment, burned animals received lactated Ringer's solution at 3,5 mL/m^2 burned area and 2 mL/kg/hr for daily maintenance.

2) The sham group (n=6) had a sham burn under anesthesia. Eighteen hours later, the animals received the diluent (0.9% NaCl) used for the endotoxin. Lactated Ringer's solution was administered at 2 mL/kg/hr for daily maintenance.

3) The treatment group (n=6) underwent the same procedure as the burn/LPS group, except for the administration of angiotensin II inhibitor Dup753 (DuPont Merck, Wilmington, DE) intravenously at 1 μg/kg immediately after burn.

Mean arterial (MAP) and central venous (CVP) pressures were measured using transducers (P231D, Statham Gould, Oxnard, CA) connected to an Electronic Medicine Honeywell Recorder (Honeywell Inc., Pleasantville, NY) for electronic calculation of mean pressures. Cardiac output (CO) was determined by the thermal dilution technique using a Swan-Ganz catheter and a cardiac output computer (Model 9520, American Edwards Laboratories, Irvine, CA).

Mesenteric arterial blood flow (Qm) was measured with a transit time ultrasonic flow probe connected to a T101 ultrasonic meter (Transonic Systems Inc., Ithaca, NY).

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

Systemic vascular resistance index (SVRI) and mesenteric vascular resistance (MVR) were calculated with the following formulas:

Cardiac index ($L/min/m^2$) = cardiac output (L/min)/body surface area

SVRI ($dyne \cdot sec \cdot cm^{-5} \cdot m^2$) = ([mean arterial pressure – central venous pressure] × 80)/cardiac index

MVR ($dyne \cdot sec \cdot cm^{-5}$) = ([mean arterial pressure – central venous pressure] × 80)/mesenteric arterial blood flow

Systemic oxygen delivery (DO_2), systemic oxygen consumption (VO_2), mesenteric oxygen delivery (mDO_2), and mesenteric oxygen consumption (mVO_2) were calculated as follows:

DO_2 ($mL/min/m^2$) = cardiac index × arterial oxygen content × 10

VO_2 (mL/min/m^2) = cardiac index × (arterial oxygen content – mixed venous oxygen content) × 10

mDO_2 (mL/min) = mesenteric arterial blood flow × arterial oxygen content/100

mVO_2 (mL/min) = mesenteric arterial blood flow × (arterial oxygen content – mesenteric oxygen content)/100

Arterial oxygen content (mL/dL) equals (Hb × 1.34) SaO_2 + (PaO_2 × 0.0031); mixed venous oxygen content (mL/dL) equals (Hb × 1.34) SvO_2 + (PvO_2 × 0.0031); mesenteric oxygen content (mL/dL) equals (Hb × 1.34) SmO_2 + (PmO_2 × 0.0031).

At the end of the 42 hours, the animals were anesthetized with 10 mg/kg intravenous ketamine and killed with 5 mL intravenous saturated KCl.

The data are presented as mean ± SEM. Within-group analysis was performed by the analysis of variance (ANOVA) for repeated measurements with the Dunnet post hoc test. Between-groups analysis was performed by ANOVA for factorial analysis with the bonferroni post hoc test. $P<0.05$ was considered statistically significant.

Results

Systemic Hemodynamics:

Baseline hemodynamic measurements were similar in all groups. All animals survived the study period (Fig. 1 and Fig. 2).

Throughout the experiment, sham animals maintained their systemic (Fig.1 and Fig. 2) and mesenteric (Fig. 4 and Fig. 5) hemodynamics within baseline range.

After thermal injury, cardiac output (CO) showed a slight increase during the first 6 hours, returning to baseline 8 hours after burn (Fig. 2). This increase was associated with a concomitant fall in the systemic vascular resistance, whereas the systemic vascular resistance index (SVRI) decreased to 78% of baseline (Fig. 2). No significant differences were observed between the 3 groups (Fig. 1) in mean arterial pressure (MAP) and central venous pressure (CVP).

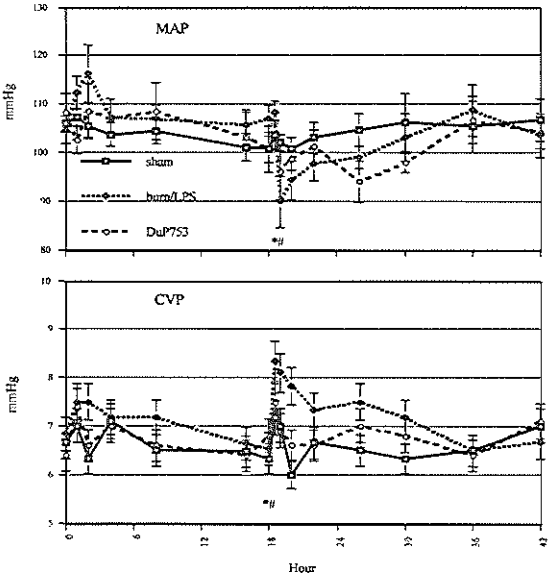

Figure 1. Systemic hemodynamic parameters: mean arterial pressure (MAP) and central venous pressure (CVP) after 40% TBSA third-degree burn (0 hour) and endotoxin administration (18 hours after burn). The angiotensin II inhibitor DuP753 was administered intravenously immediately after burn. Differences were not statistically significant. $P<0.05$. * vs. baseline, # vs. sham, ¥ vs. DuP753.

After LPS administration, a typical biphasic response was observed. The hemodynamic alteration was more pronounced during the second phase: after a marked

drop in CO to 77% of baseline, a hyperdynamic period began 8 hours after endotoxin administration (Fig. 2). At this time point, SVRI dropped to 69% of baseline. MAP showed a 14% decrease immediately after LPS infusion in both burn/LPS groups. 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 (Fig. 1 and Fig. 2).

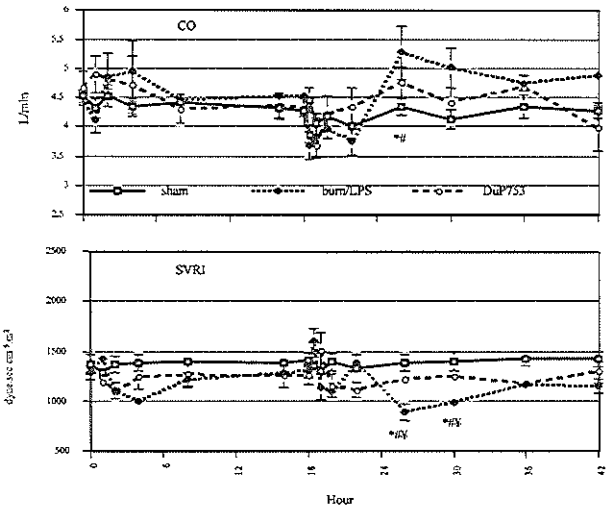

Figure 2. Systemic hemodynamic parameters: cardiac output (CO) and systemic vascular resistance index (SVRI) after burn (0 hour) and endotoxin administration (18 hours). The hemodynamic alteration was more pronounced after LPS infusion. DuP753 had a positive impact on CO and SVRI. $P<0.05$, * vs. baseline, # vs. sham, ¥ vs. DuP753.

Mesenteric Hemodynamics:

Although cardiac output did not fall after thermal injury, mesenteric arterial blood flow (Qm) decreased significantly to approximately 58% of baseline during the first 4 hours after burn (Fig. 3). In contrast to the previously observed postburn reduction in systemic vascular resistance, mesenteric vascular resistance (MVR) showed a significant increase (201% of baseline) during the early postburn phase (Fig. 4). During the late postburn phase, starting 4 hours after insult, a mesenteric reperfusion phase became manifested: Qm increased by 44% over baseline.

Compared with burned animals not receiving the angiotensin II antagonist, DuP753-treated animals showed no reduction in Qm after burn; in fact, it increased by 33% over baseline during the early postburn phase (Fig. 3). Animals in the DuP753 treatment group maintained, unlike the nontreated burned animals, a stable MVR near baseline during this early mesenteric vasoconstrictive phase (Fig. 4).

At 18 hours after burn, mesenteric hemodynamic measurements were comparable to baseline levels in all groups.

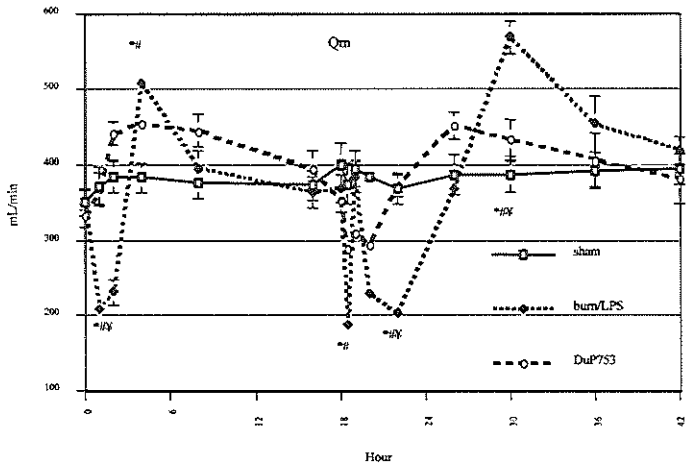

Figure 3. Burn (0 hour) and endotoxin administration (18 hours after burn) significantly reduced mesenteric arterial blood flow (Qm). Treatment with DuP753 prevented the impact of burn/sepsis on Qm. $P<0.05$, * vs. baseline, # vs. sham, ¥ vs. DuP753.

Administration of LPS to burned animals resulted in a biphasic response of mesenteric ischemia and reperfusion. The second insult yielded a significant mesenteric vasoconstriction, with an increase of MVR to 222% of baseline during the first 8 hours after LPS infusion.

Similarly, Qm decreased significantly to 53% of baseline during the same time of peak MVR. After an initial moderate drop in Qm by 13% of baseline, burned animals treated with DuP753 showed no signs of mesenteric vasoconstriction after the second insult (LPS): no marked increase was noticed in MVR. Compared with nontreated animals,

DuP753 treatment resulted in a significant improvement of Qm after the second impact (LPS); 4 hours after LPS infusion, Qm reached 112 % of baseline in the DuP753 group, whereas the flow was still significantly reduced to 57% of baseline in the burn/LPS group (Fig. 3 and Fig. 4).

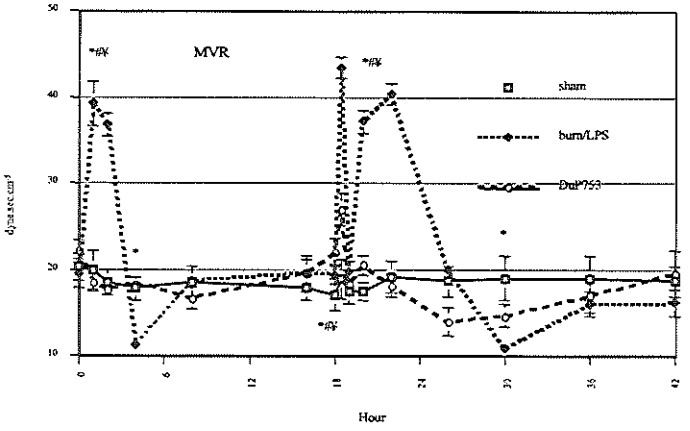

Figure 4. Changes in mesenteric vascular resistance (MVR) after burn (0 hour) and endotoxin administration (18 hours). $P<0.05$, * vs. baseline, # vs. sham, ¥ vs. DuP753.

Systemic Oxygen Delivery and Consumption:

After an initial reduction, systemic oxygen delivery (DO_2) and oxygen consumption (VO_2) showed a marked increase during the first 4 hours after burn (Fig. 5).

Administration of LPS yielded a significant drop in DO_2 during the first hour. VO_2 remained unchanged at this time point. Animals treated with DuP753 remained at baseline after LPS infusion (Fig. 5). During the post-LPS hyperdynamic phase, both DO_2 and VO_2 were increased.

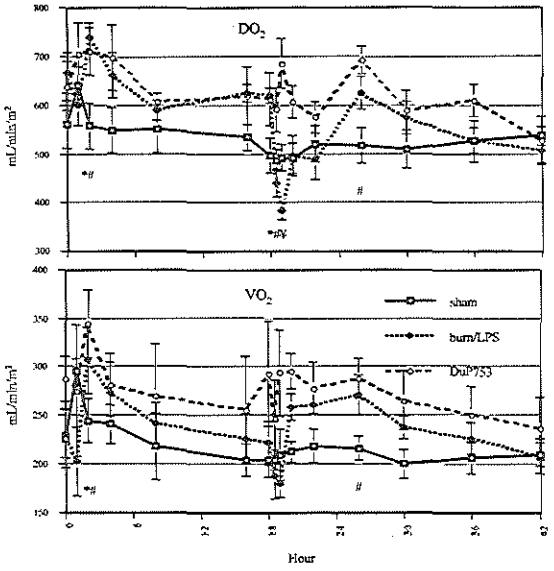

Figure 5. Effects of burn (0 hour) and endotoxin administration (18 hours) on systemic oxygen delivery (DO_2) and oxygen consumption (VO_2). $P<0.05$, * vs. baseline, # vs. sham, ¥ vs. DuP753.

Mesenteric Oxygen Delivery and Consumption:

During the first 2 hours after burn, both mesenteric oxygen delivery (mDO_2) and mesenteric oxygen consumption (mVO_2) showed a significant decrease to 60% and 53% of baseline, respectively. In contrast, animals treated with DuP753 showed an improvement in postburn mDO_2 and mVO_2 to 128% and 112% of baseline, respectively (Fig. 6).

The second insult (LPS) yielded a dramatic reduction in mDO_2 to 51% of baseline. Postburn treatment with DuP753 prevented this impact of LPS, and mDO_2 remained at baseline (Fig. 6). Similarly, after LPS infusion, reduced mVO_2 was improved in animals receiving DuP753 (58% vs. 127% of baseline).

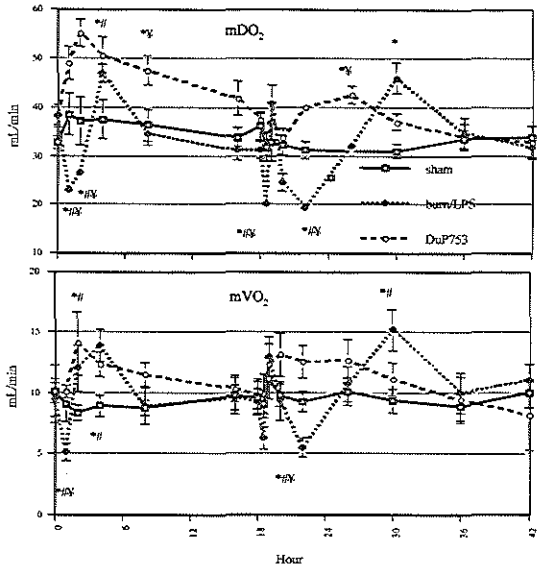

Figure 6. Mesenteric oxygen delivery (mDO_2) and oxygen consumption (mVO_2) were significantly reduced after burn (0 hour) and endotoxin administration (18 hours after burn). These detrimental effects were ameliorated by DuP753 treatment. $P<0.05$, * vs. baseline, # vs. sham, ¥ vs. DuP753.

Discussion

Recently, it has become apparent that mesenteric vasculature reacts in a very sensitive way to injury. A clinical review of the autopsy records of severely burned patients revealed a 61% incidence of intestinal ischemia.[15] At the time of death, sepsis was manifested in more than 80% of these patients.

The relation between gut ischemia and sepsis remains to be defined. Splanchnic ischemia has been reported to alter the intestinal mucosal barrier, leading to the escape of indigenous bacteria and their endotoxins to the systemic circulation and remote body organs.[7,16,17] Recently, the role of the gut as a cytokine-generating organ in the post-injury inflammatory response has been studied.[18] As the causal relation of bacterial translocation or gut-derived cytokines to the development of sepsis and multiple organ dysfunction syndrome has become more apparent and more complex, the need to identify the mechanisms involved in the pathophysiology of post-injury gut dysfunction is increasing.

On the other hand, endotoxin (gut-derived or due to other factors), has been reported to act synergistically to promote mucosal damage and bacterial translocation after major burn injuries.[13] Since patients with major burn injuries appear to be frequently subjected to repeated episodes of endotoxemia,[12] the impact of the second insult, which is believed to have more dramatic consequences, may be responsible for the high morbidity and mortality rates in this group. Therefore, we postulated that a treatment modality abrogating mesenteric ischemia after the first injury would decrease the impact of the second hit (if occurred).

Our current data confirm those of previous studies reporting the alteration of mesenteric hemodynamics secondary to thermal injury.[19,20] Despite indicators of adequate resuscitation (i.e. minor changes in cardiac output, mean arterial pressure and central venous pressure), a significant reduction in mesenteric arterial blood flow (Qm) was observed in this study during the early phase after thermal injury. Thus, altered systemic hemodynamics could not be solely responsible for the observed postburn mesenteric vasoconstrictive phase. The significant increase in mesenteric vascular resistance (MVR) during the first 4 hours after burn, despite the marked decrease in

systemic vascular resistance, suggests the involvement of certain mediators selectively acting on mesenteric vasculature.

The renin-angiotensin axis appears to play a critical role in the pathophysiology of thermal injury. Hilton et al[14] reported a 4-fold increase in angiotensin II levels 6 hours after thermal injury. Angiotensin II is a potent vasoconstrictor that exhibits important mesenteric selectivity, thought to be caused by an increased affinity of the angiotensin II receptors on the splanchnic vascular smooth muscle.[21] In a pig model of cardiogenic shock, a disproportionate mesenteric ischemia resulting from selective splanchnic vasospasm was observed.[22] Ablation of the renin-angiotensin axis was shown to abolish this response. Moreover, the observed hemodynamic changes were found to correlate with serum angiotensin II concentrations and could be reproduced in the absence of shock by the infusion of angiotensin II.[23]

The data of this study clearly show that postburn treatment with DuP753, a specific angiotensin II receptor antagonist,[24] can prevent postburn vasoconstriction and subsequently abrogate the impact of postburn endotoxemia on superior mesenteric artery blood flow (Qm). In contrast to an approximately 50% decrease in Qm occurring as early as the first hour after thermal injury, an initial increase by 11% in Qm was observed after DuP753 treatment. Postburn administration of DuP753 was found to prevent burn-induced mesenteric vasoconstriction: MVR remained near baseline in treated animals, compared with an almost 100% increase in nontreated animals.

Systemic oxygen delivery (DO_2) and oxygen consumption (VO_2) showed a pattern of increase similar to that seen in cardiac output (CO) after thermal injury. In contrast, mesenteric oxygen supply (mDO_2) was significantly reduced during this early postburn phase. This observation also confirms the independence of the postburn altered mesenteric circulation and oxygenation, with respect to the systemic circulation.[20,25,26] At the same time, mesenteric oxygen consumption (mVO_2) showed a reduction that was dependent on the mDO_2. This postburn pathologic supply-dependent gut oxygen consumption was observed before in experimental models. [20,27] This early decreased mVO_2 indicates the inability of the gut to compensate for inadequate oxygen delivery by increasing oxygen extraction, resulting in tissue hypoxia. Early hypoxia in the splanchnic

region is suggested as a plausible mechanism behind the development of secondary organ failure, especially in sepsis. [28] Animals receiving DuP753 showed no decrease in mDO_2 after thermal injury. Administration of DuP753 after thermal injury resulted in a significant improvement in mDO_2 2 hours after burn; 49.14 mL/min [DuP753] vs. 23.03 [burn], $P<0.05$). Postburn mesenteric hypoxia, as indicated by a decreased mVO_2, was not observed in animals in the treatment group. The action of DuP753 appears to be selective. The enhancement in oxygen supply to meet the increased oxygen demand was noticed only in the mesenteric circulation: no differences were found between treated and untreated burned animals with respect to DO_2 or VO_2.

Although no important changes were noticed in DO_2 and VO_2 in both burn/LPS and DuP753 groups after endotoxin infusion, the impact of this second insult on the mesenteric oxygenation was dramatic. A typical biphasic response was observed, with a more pronounced flow-dependent hypoxic period. The first 8 hours after LPS administration to burned animals were marked by a significant decrease in mDO_2. Mesenteric oxygen consumption showed a pathologic dependence on oxygen delivery, leading to an oxygen debt that limits metabolism. LPS alone has been shown to cause such an alteration in mesenteric oxygenation.[26] These results could be explained by a defect in microvascular regulation of blood flow that interfered with the optimal distribution of a limited supply of oxygen in accordance with tissue oxygen needs.[29] Our data demonstrate that these negative effects of LPS on mesenteric oxygenation can be prevented by DuP753 treatment. After LPS challenge, burned animals in the treatment group showed a significant improvement in their mesenteric oxygenation status compared with nontreated animals (mVO_2/mDO_2, 4 hours after LPS infusion: 12.57/40.08 mL/min [DuP753] vs. 5.47/19.37 [burn], $P<0.05$).

Conclusion

In our combined burn and sepsis chronic-instrumented porcine model, both insults had adverse effects on mesenteric circulation and oxygenation, whereas the impact of the second insult was more pronounced and prolonged. Angiotensin II appears to be involved in this process. Postburn treatment with DuP753, a specific angiotensin II receptor antagonist, can ameliorate burn- and endotoxemia-induced mesenteric ischemia by improving mesenteric blood flow and oxygen supply.

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CHAPTER 5

Angiotensin II Inhibitor DuP753 Attenuates Burn- and Endotoxin-Induced Lipid Peroxidation, Mucosal Permeability and Bacterial Translocation

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Abstract

Objective: To investigate whether treatment with angiotensin II inhibitor DuP753 can attenuate mucosal injury and bacterial translocation in a burn/endotoxemia chronic porcine model.

Summary Background Data: Thermal injuries and endotoxemia have been shown to induce ischemia and reperfusion injury to the intestine, leading to increased mucosal permeability and bacterial translocation. Recently, angiotensin II inhibitor DuP753 has been reported to ameliorate burn- and sepsis-induced gut ischemia.

Methods: Twenty-one female pigs were anesthetized and 14 of them received 40% total body surface area third-degree burn. DuP753 was administered intravenously at 1 μg/kg to 7 pigs immediately after burn. Eighteen hours after burn, 100 μg/kg *Escherichia coli* lipopolysaccharide (LPS) was administered intravenously. Plasma conjugated dienes (PCDs), an index of lipid peroxidation, were measured every 6 hours. Intestinal permeability was assessed every 6 hours by measuring the lactulose/mannitol (L/M) excretion ratio. At the end of the study (42 hours), tissue samples were harvested for bacteriologic cultures.

Results: PCD levels were significantly elevated 8 hours after burn (182% increase vs. sham, $P<0.01$). LPS caused a higher and prolonged increase in PCD levels (210% of baseline). Treatment with DuP753 significantly reduced PCD levels after burn and after LPS ($P<0.01$). Intestinal permeability, as assessed by the L/M ratio, showed 6- and 12-fold increases after thermal injury and LPS, respectively ($P<0.05$). In contrast, the L/M ratio was only doubled in DuP753-treated animals ($P<0.01$). Bacterial translocation was significantly increased after burn and endotoxin (6/7 vs. 1/7 sham, $P<0.05$). The incidence of bacterial translocation in the DuP753-treated animals was similar to that in the sham group (2/7).

Conclusions: Angiotensin II appears to play a pivotal role in burn- and endotoxin-induced intestinal ischemia and reperfusion injury, with subsequent increases in permeability and bacterial translocation. Postburn administration of angiotensin II receptor antagonist DuP753 significantly reduces the extent of these events.

Introduction

After major thermal injuries, elevated serum endotoxin levels have been documented in humans.[1,2] Endotoxin levels were found to increase within 24 hours after injury [2], peaking on days 3 and 4 after burn.[1]

Previous studies have investigated the impact of thermal injuries or endotoxin on intestinal mucosal integrity.

The association between thermal injuries and the dysfunction of the intestinal mucosal barrier has been clearly documented. A significant increase in intestinal permeability in thermally injured patients was reported within 24 hours and throughout the first 2 weeks after injury.[3,4] This alteration in the mucosal barrier may contribute to the translocation of indigenous bacteria and endotoxins into the circulation.[5] In turn, endotoxin has been shown to impair gut barrier function in humans: a brief exposure to circulating endotoxin was reported to increase the permeability of the normal gut.[6] The cause and the clinical significance of the altered intestinal mucosal integrity under such conditions remain unclear. Based on experimental studies, intestinal ischemia and reperfusion injury appears to be the most likely mechanism underlying this phenomenon.[7] In a previous study, angiotensin II was shown to be one of the mediators responsible for the impairment of mesenteric blood flow and oxygenation after burn and endotoxemia.[8] Pharmacological blockade of angiotensin II receptors by a specific agent (DuP753) was found to abolish these events.

Therefore, we investigated in this study whether treatment with angiotensin II antagonist DuP753 can abrogate the detrimental impact of burn and endotoxin on mucosal barrier function in a chronic porcine burn/endotoxemia model.

Methods

The following experimental protocols were approved by the Animal Care and Use Committee of the University of Texas Medical Branch (ACUC # 90-09-103).

Surgical preparation

Studies were performed in 21 female mini-pigs (weight 20-25 kg). After an overnight fast, the pigs were sedated with intramuscular ketamine (10 mg/kg) and mechanically ventilated with 2% to 2.5% halothane after endotracheal intubation. Through a neck incision, a catheter was placed via the right common carotid artery into the abdominal aorta, and a Swan-Ganz thermal dilution catheter (Model 93 A-131-5F, American Edwards Laboratories, Anasco, PR) was positioned in the pulmonary artery via the right jugular vein. A Witzel jejunostomy was performed using a 12F Foley catheter, and a 12F Foley catheter was inserted in the urinary bladder.

Experimental design

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

The pigs were randomized into 3 groups:

1) The burn/lipopolysaccharide (LPS) group (n=7) had a 40% total body surface area (TBSA) third-degree flame burn under general anesthesia as described above. The pigs were resuscitated according to the Parkland formula and received lactated Ringer's solution (4 mL/kg/% TBSA burned) starting immediately after the burn; half was given in the first 8 hours after burn and the remainder in the next 16 hours. Eighteen hours after burn, 100 μg/kg *E. coli* LPS (0111:B4; Difco, Detroit, MI) was administered intravenously. During the second day of the experiment, the burned animals received lactated Ringer's solution at 3,5 mL/m^2 burned area and 2 mL/kg/hr for daily maintenance.

2) The sham group (n=7) had a sham burn under anesthesia. Eighteen hours later, the animals received the diluent (0.9% NaCl) used for the endotoxin. Lactated Ringer's solution was administered at 2 mL/kg/hr for daily maintenance.

3) The treatment group (n=7) underwent the same procedure as the burn/LPS group, except for the administration of angiotensin II inhibitor DuP753 (DuPont Merck, Wilmington, DE) intravenously at 1 μg/kg immediately after burn.

Mean arterial (MAP) and central venous (CVP) pressures were measured using transducers (P231D, Statham Gould, Oxnard, CA) connected to an Electronic Medicine Honeywell Recorder (Honeywell Inc.,Pleasantville, NY) for electronic calculation of mean pressures. Cardiac output (CO) was determined by the thermal dilution technique using a Swan-Ganz catheter and a cardiac output computer (Model 9520, American Edwards Laboratories, Irvine, CA).

Blood samples for plasma conjugated diene (PCD) assays were taken from the arterial line catheter at baseline and then every 6 hours, beginning 1 hour after burn.

Permeability assessment

After the animals had recovered from the surgical instrumentation, a solution of 10 g lactulose and 5 g mannitol, diluted in 60 mL distilled water (1,160 mOsm/kg), was given via the jejunostomy tube, and urine was collected for a 6-hour period to obtain baseline measurements. Lactulose/mannitol (L/M) assessment was repeated every 6 hours. At the completion of collection, the urine was divided into aliquots and frozen at 20°C until assayed. Urinary lactulose and mannitol concentrations were simultaneously determined by the technique described by Fleming et al,[9] using high- pressure liquid chromatography coupled with pulsed amperometric detection (HPLC-PAD). Urine was diluted 2- to 20-fold with deionized water, depending on the collection volume. One mL diluted urine was mixed with internal saccharide standards, desalted, vortex-mixed, centrifuged, and filtered. Fifty microliters of the filtrate was injected onto a 250 x 40 mm anion exchange column (Dionex Carbopak, PAI, Houston, TX) and eluted with 0.15 mol/L NaOH, 1 mL/min, at 20°C. Detection was by pulsed amperometric detection with a working gold electrode and silver/silver chloride reference electrode, with a detection potential of +0.05, oxidation potential of +0.06, and a reduction potential of -0.95V. Quantification was by peak height analysis and peak height ratios, with internal standardization. This method offers excellent separation of the carbohydrates and precise detection at low concentrations (lactulose 0.3 mg/L). The amount of each sugar excreted in the urine during 6 hours was then converted to a percentage of the amount of the given

sugar, reflecting the excretion fraction of each sugar. By dividing the lactulose and mannitol excretion fractions, a permeability index- the lactulose/mannitol (L/M) ratio- was calculated.

Conjugated diene assay

Plasma conjugated dienes (PCDs) were measured according to the method of Till et al.[10] Conjugated dienes were extracted from the plasma by using a 2:1 (vol/vol) mixture of chloroform and methanol. Seven mL of the chloroform/methanol mixture, preheated to 45°C, was added to 0.5 mL plasma. The mixture was then vigorously agitated for 2 minutes and centrifuged for 5 minutes at 3,000 rpm at 4°C. The lower layer was aspirated and pipetted into a test tube and dried under a direct flow of nitrogen gas. The residue was reconstituted with 1.5 mL heptane and read on a spectrophotometer at 233 nanometers (Spectronic 1001; Milton Roy Co., Houston, TX).

Testing for bacterial translocation

At the end of the 42 hours, the animals were anesthetized with 10 mg/kg intravenous ketamine and killed with 5 mL intravenous saturated KCl. Using aseptic technique, through a midline laparotomy incision, peritoneal fluid and tissue samples from the proximal and distal mesenteric lymph nodes, spleen, liver, kidney, lung, jejunum, ileum, cecum, and colon were taken for bacteriologic cultures. Collected tissue samples were weighed, and 0.5 g of each was homogenized in a tissue grinder with 4.5 mL nonbacteriostatic saline to create a 1:10 dilution of the original sample; 0.1 mL and 0.01 mL (of the 1:10 dilution) were inoculated onto a MacConkey agar plate and a Columbia Nutrient Agar (CNA) plate for isolation of gram-negative and gram-positive organisms, respectively. Therefore, one colony would represent 1×10^2 and 1×10^3 colony-forming units per gram of tissue, respectively, for each inoculum size. Limits of detection were 100 organisms per gram of tissue. Inoculated plates were incubated at 37°C for 24 and 48 hours and read with a Darkfield Quebec Colony Counter (Model 3330, American Optical Co., Buffalo, NY). Cultures were considered positive when more than 100 colonies per gram of tissue were found. All bacterial isolates were identified by biotype using a Microscan 4 bacterial analyzer (Baxter, Sacramento, CA.)

Statistical analysis

The data are presented as mean ± SEM. Within-group analysis was performed by the analysis of variance (ANOVA) for repeated measurements with the Dunnet post hoc

test. Between-groups analysis was performed by ANOVA for factorial analysis with the Bonferroni post hoc test. Bacteriologic tissue culture results were analyzed by the Fischer exact test. $P < 0.05$ was considered statistically significant.

Results

Systemic hemodynamics

Baseline hemodynamic measurements were similar in all groups. All animals survived the study period (Fig. 1).

After thermal injury, the cardiovascular variables (CO, MAP and CVP) were stable as a result of adequate resuscitation (Parkland formula). After the administration of endotoxin there were some moderate hemodynamic changes, but of no statistical significance (Fig. 1).

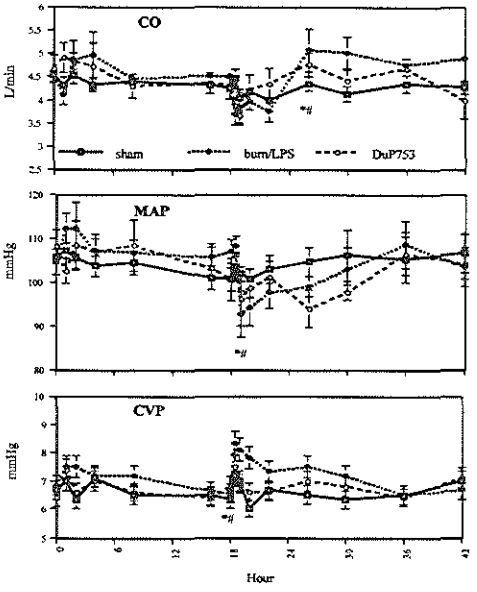

Figure 1. Systemic hemodynamic parameters: cardiac output (CO), mean arterial pressure (MAP), and central venous pressure (CVP) after 40% TBSA third-degree burn (0 hour) and endotoxin administration (*E. coli* LPS, 18 hours after burn). DuP753 was administered intravenously immediately after burn. Differences were not statistically significant. $P<0.05$, *vs. Baseline, # vs. Sham, ¥ vs. DuP753.

Conjugated diene assay

Levels of PCDs were significantly increased in the burn/LPS group at 4 and 8 hours after burn (Fig. 2). Postburn PCDs reached 168% of baseline at 4 hours, and the differences compared with sham animals were significant. Eight hours after thermal

injury, PCDs showed a 182% increase compared with control animals. DuP753 treatment resulted in a significant decrease of postburn PCDs at 4 hours (Fig. 2).

LPS infusion to burn animals yielded a higher and prolonged elevation of PCDs, showing an increase of 178% of baseline at 4 hours and 210% of baseline at 8 hours after LPS infusion. PCD levels remained significantly elevated in the burn/LPS group until the end of the study period. In contrast, animals treated with DuP753 showed no elevation in their PCD levels after LPS administration (Fig. 2).

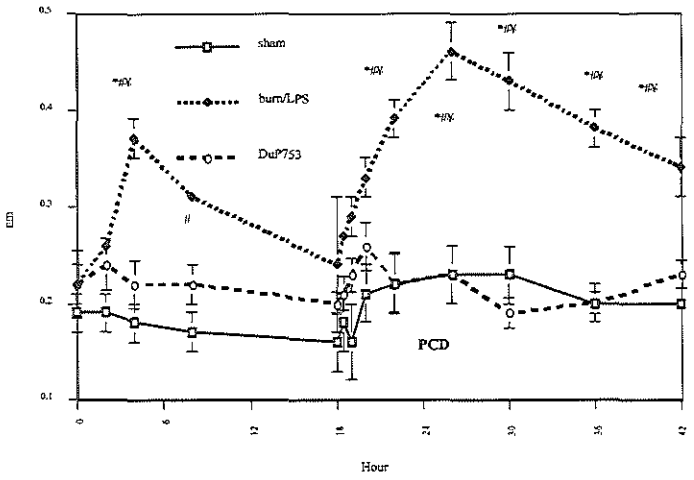

Figure 2. Plasma conjugated diene (PCD) levels, an index of ischemia and reperfusion injury-induced lipid peroxidation, were significantly elevated after burn (0 hour) and endotoxin administration (18 hours after burn). Postburn treatment with DuP753 yielded a significant reduction in PCD levels after burn and endotoxin challenges. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. DuP753.

Lactulose and mannitol assay

There were no differences between groups in baseline levels of the L/M ratio (Fig. 3). The L/M ratio for burned animals was moderately increased at 6 hours after burn. Intestinal permeability, as indicated by the L/M ratio, showed a 6-fold increase at 12 hours after burn in the burn/LPS group compared with only a doubled increase in the treatment group. The differences between groups were significant at this time point (Fig. 3).

The changes in mucosal permeability, as measured by the L/M ratio, were more pronounced after LPS administration to burned animals. The L/M excretion ratio was 12- and 10-fold elevated in the burn/LPS group at 12 and 18 hours after LPS, respectively. In contrast, treated animals showed only a 3- and 2-fold increase at these time points. Differences between groups were significant (Fig. 3).

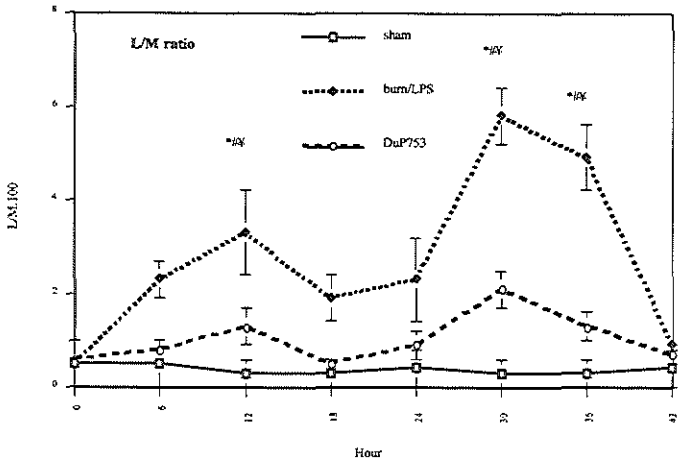

Figure 3. Changes in intestinal permeability, as assessed by the lactulose/mannitol (L/M) excretion ratio, after 40% TBSA third-degree burn (0 hour) and endotoxin administration (*E. coli* LPS, 18 hours after burn). DuP753 was administered intravenously immediately after burn. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. DuP753.

Quantitative bacteriologic culture of tissue samples

The rates of positive tissue cultures were significantly higher in animals receiving burn and LPS compared with the sham group (6/7 vs.1/7, $P < 0.05$). Animals treated with DuP753 showed rates of positive tissue cultures comparable to those of the control animals (Fig. 4). Specific tissue isolates and their origin from animals in the three groups are shown in Table 1. Only tissue cultures that were positive with enteric bacteria of the same biotype as that found in the intestine of corresponding animal were interpreted as evidence of bacterial translocation. In the burn/LPS group, 86% of the animals showed bacterial translocation to the mesenteric lymph nodes (MLNs) and lung, 71.4% to the

spleen and liver, and 43% to the kidney (Fig. 4). In contrast, only 14% of the treated animals showed bacterial translocation to the MLNs and lung (P<0.05), 28% to the liver, 14% to the spleen (P=0.053), and none to the kidney
(Fig. 4).

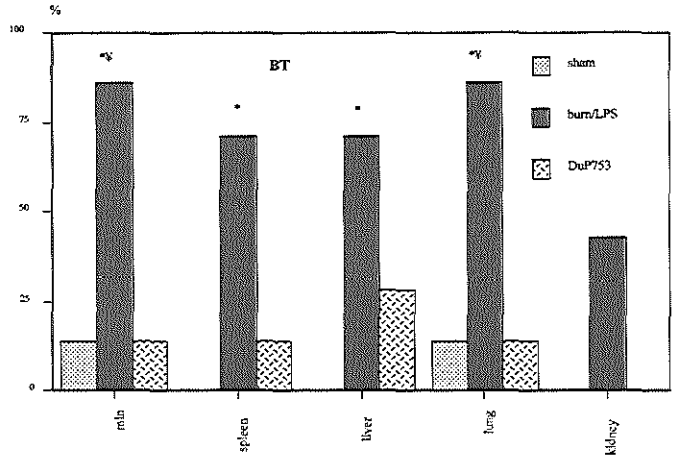

Figure 4. Burn and endotoxin resulted in a significant increase in the rates of positive tissue cultures with enteric bacteria. The incidence of bacterial translocation to remote organs was significantly reduced in animals treated with DuP753. Data are presented as percentages of harvested tissue samples. P <0.05, * vs. baseline, # vs. sham, ¥ vs. DuP753.

Table 1. Rates, types, and origins of tissue isolates from the 3 study groups. Only tissue cultures that were positive with enteric bacteria of the same biotype as that found in the intestine of corresponding animal are presented. (j = jejunum, i = ileum, c = cecum).

Table 1. Specific tissue isolates from the three study groups

tissue samples	positive/total	organism	origin
		Sham	
MLN	(1/7)	Escherichia coli	i/c
		S intermedius	i/c
		Enterococcus faecium	j/c
Lung	(1/7)	S intermedius	i/c
		Burn/LPS	
MLN	(6/7)	Streptococcus bovis	j/i/c
		Staphylococcus hyicus hyicus	j/i/c
		Enterococcus fecalis	j/i/c
Spleen	(5/7)	Staphylococcus hyicus hyicus	j/i/c
		Enterococcus fecalis	j
Liver	(5/7)	Staphylococcus hyicus hyicus	j/i
		Escherichia coli	j/i/c
		Enterococcus fecalis	j/i/c
		Klebsiella pneumoniae	j/i/c
Lung	(6/7)	Staphylococcus hyicus hyicus	j/i/c
		Staphylococcus aureus	j/i/c
		Enterococcus fecalis	j/i/c
		Enterococcus faecium	j/i/c
		Streptococcus bovis	j/i/c
		Klebsiella pneumoniae	j/i/c
Kidney	(3/7)	Staphylococcus aureus	i/c
		Escherichia coli	j/i/c
		Stafhylococcus hyicus hyicus	c
		Burn/DuP753/LPS	
MLN	(1/7)	Enterococcus faecium	j/i/c
		Staphylococcus haemolyticus	j
		Escherichia coli	j/i/c
Spleen	(1/7)	Staphylococcus hyicus hyicus	i/c
Liver	(2/7)	Staphylococcus hyicus hyicus	i/c
		Enterococcus fecalis	j/i/c
Lung	(1/7)	Enterococcus faecium	j/i/c
Kidney	(0/7)		

Discussion

There is accumulating evidence that failure of the gut barrier function may play an important role in the initiation or progression of the hypermetabolic response and multiple organ dysfunction syndrome (MODS) that frequently occur after major trauma.[11,12] Although the causal relation between the gut as a reservoir of enteric bacteria and endotoxins and the development of sepsis and/or MODS remains to be defined, bacterial translocation has been associated by various pathological conditions, including thermal injury[7] and endotoxemia.[13] Intestinal ischemia with a resultant reperfusion injury appears the most likely mechanism in the pathophysiology of decreased intestinal mucosal integrity, occurring shortly after thermal injury.[7] Later in the postburn period, altered intestinal permeability seems to be related to the episodes of endotoxemia to which burned patients are frequently exposed.[1,2] In such situations, intestinal ischemia and sepsis can become self-sustaining because endotoxin, gut- or tissue-derived, has been documented to decrease intestinal blood flow and promote bacterial translocation.[14-16] In addition to the elucidation of the molecular mechanisms underlying the injury of sepsis-induced intestinal ischemia, the identification of these mediators may facilitate the development of interventions to modulate the mesenteric blood flow and prevent subsequent pathological events. Therefore, we recently reported on possible mediators in this complex process. The results of our previous study [8] have clearly shown that angiotensin II plays a critical role in the process of mesenteric vasoconstriction triggered by burn and endotoxin. Postburn treatment with DuP753, a specific angiotensin II receptor antagonist, was found to ameliorate burn- and endotoxin-induced mesenteric ischemia by improving mesenteric blood flow and oxygen supply. We therefore hypothesized that DuP753 treatment can attenuate burn- and endotoxin-induced gut barrier failure.

In the current study, the intestinal permeability was measured by assessment of the lactulose/mannitol (L/M) excretion ratio. Mannitol, a monosaccharide, has a radius of 0.4 nm and is absorbed transcellularly through aqueous pores in the cell membrane. Lactulose, on the other hand, is larger (0.52 nm) and its absorption occurs via the paracellular pathway through damaged tight junctions. When absorbed, both sugars cross the gut to the circulation, remain unmetabolized, and are excreted by the kidney.

Intestinal permeability, as assessed by the L/M ratio, was 6-fold increased 12 hours after thermal injury. These results are in agreement with those of previous reports. Deitch [3] documented a 3-fold increase in intestinal permeability in thermally injured patients during the first 24 hours after injury with use of the L/M ratio. Postburn administration of the angiotensin II antagonist DuP753 resulted in a significant reduction of L/M ratios in burned animals. This treatment effect on postburn intestinal permeability may be beneficial in reducing the susceptibility to infection in patients with thermal injuries, because a relation between increased permeability and postburn infections has been reported in humans.[4,17] Intestinal permeability was studied in burned patients during the first 2 weeks by measuring the L/M ratio.[4] There was a clear correlation between the increased L/M ratio on postburn day 2 and the occurrence of significant clinical infections during the first 2 postburn weeks. Ziegler et al[17] studied patients 2 weeks after injury by the L/M ratio and noted that only infected burned patients had altered permeability. However, their patients were studied in the late postburn period, during which other factors, such as endotoxemia, could have contributed to the reported altered permeability. Endotoxin alone has been reported to increase intestinal permeability in humans.[6] Thus, it appears that thermally injured patients are exposed to two episodes of increased permeability: first, the initial burn trauma, and second, the insult of endotoxemia. In our model of combined burn/LPS, the impact of endotoxin, administered 18 hours after burn, on the intestinal permeability was dramatic. Intestinal permeability, as indicated by the L/M ratio, showed a significant and prolonged increase in burned animals receiving LPS (12- and 10-fold increase at 12 and 18 hours after LPS infusion, respectively). The postburn blocking of angiotensin II significantly reduced these detrimental effects of postburn endotoxemia on intestinal permeability.

Because the permeability was increased in the burn/LPS group, the incidence of positive tissue cultures with enteric bacteria was also significantly elevated. Eighty-six percent of the animals with combined burn and LPS insults showed bacterial translocation. The linear correlation between both the increased permeability index and bacterial translocation rate in these animals suggests a causal relationship. Another finding in our study that may confirm this relation is the observation of a significant decrease in bacterial translocation rates in the treatment group. The bacterial

translocation rates of animals receiving burn and LPS and treated with DuP753 and those of the sham animals did not differ significantly (14-28% vs. 14%). The involvement of angiotensin in the occurrence of bacterial translocation after burn and bacterial challenge has recently been reported.[18] Mice pretreated with angiotensin converting enzyme inhibitor showed a lower incidence of bacterial translocation and a higher survival rate after thermal injury and bacterial challenge compared with control animals. In the current study, a more target-specific angiotensin-blocking agent was given, as a treatment modality, immediately after the first insult "burn".

The exact mechanism(s) by which major thermal injuries and endotoxemia affect the intestinal permeability and bacterial translocation remain(s) to be elucidated. However, as previously suggested, ischemia and reperfusion injury appears to play a pivotal role in this complex process. The resultant oxygen-derived free radicals have been implicated in the subsequent tissue damage that results in intestinal mucosal injury .[6,19,20] Although oxidant-induced damage may involve many cell components, peroxidative decomposition of membrane lipids has been considered as the basis of cell injury.[21] According to Tribble et al, [21] lipid peroxidation is important in oxidative injury because it increases the number of free radical chain reactions, compromises detoxification systems, and causes direct deleterious effects, because lipid peroxidation products themselves are considered toxic. Lipid peroxidation, initiated by hydroxyl radicals, results in the formation of lipid-derived free radicals such as conjugated dienes, lipid hydroperoxide radicals, and lipid hydroperoxides.[19] Measurement of conjugated dienes is frequently used as an index of lipid peroxidation. In the current study, plasma conjugated diene (PCD) concentrations showed a 68% increase at 4 hours after burn and a return to baseline at 18 hours after burn. After LPS infusion, the elevation of PCDs was augmented and prolonged, beginning as early as 2 hours after LPS infusion, peaking (210% of baseline) at 8 hours after LPS infusion, and remaining elevated until the end of the study period. Administration of DuP753 directly after thermal injury yielded a significant reduction in postburn reperfusion injury, as indicated by the PCD levels. Treated animals showed no significant increase in their PCD levels compared with baseline and sham animal levels. From these data, we cannot determine whether the PCDs are solely the product of intestinal ischemia and reperfusion injury. These radicals

could have their origins from other organs, such as the liver, burned skin, and lung.[22,23] However, the observation that systemic hemodynamic parameters (cardiac output, mean arterial pressure, and central venous pressure) showed no significant changes because of adequate resuscitation, together with the data of our previous study [8] in which mesenteric blood flow was significantly reduced under the same conditions, argue for the concept that the gut is, at least in part, a major contributor to these lipid-derived free radicals. The beneficial effect of DuP753 on mesenteric arterial blood flow and mesenteric oxygenation after burn and endotoxin challenges, seen in our previous study [8], may explain the action of DuP753 treatment in attenuating burn and endotoxemia reperfusion injury, as assessed by PCD measurements in the current study.

In conclusion, major thermal trauma appears to cause ischemia and reperfusion injury, with a subsequent increase in intestinal permeability and bacterial translocation, all of which are augmented by a second insult of endotoxemia. The data of this study indicate that angiotensin II plays a central role in this process. Blocking angiotensin II receptor by DuP753 can attenuate these detrimental effects of burn and endotoxin.

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Effects of Interleukin-1α Administration on Intestinal Ischemia and Reperfusion Injury, Mucosal Permeability and Bacterial Translocation in Burn and Sepsis

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Abstract

Objective: To evaluate the effect of interleukin-1α (IL-1α) on the mesenteric circulation, intestinal mucosal integrity, and bacterial translocation in a burn/endotoxemia chronic porcine model.

Summary Background Data: Major burn and sepsis are associated with a high mortality, ischemia/reperfusion injury to the intestine, and an increased rate of bacterial translocation. Pathologic alterations of IL-1 synthesis, degradation, and binding to receptors have been reported. Manipulation of IL-1-mediated effects might be of therapeutic utility in the future.

Methods: Twenty-one female pigs were instrumented with an ultrasonic flow probe on the superior mesenteric artery and a catheter into the superior mesenteric vein. After 5 days, all animals were anesthetized, and 14 received 40% total body surface area third-degree burn. IL-1α was administered intravenously at 1000 ng/kg to 7 pigs immediately after burn. Eighteen hours after burn, 100 μg/kg *Escherichia coli* lipopolysaccharide (LPS) was administered intravenously. Systemic and splanchnic hemodynamics were measured and blood samples were drawn for blood gas analysis. Intestinal permeability was assessed every 6 hours by measuring the lactulose/mannitol (L/M) excretion ratio. At the end of the study (42 hours), tissue samples were harvested for bacteriologic cultures.

Results: Mesenteric blood flow was significantly decreased after burn and endotoxin to 59% and 60% of baseline, respectively. Administration of IL-1α significantely improved mesenteric blood flow postburn and post-LPS (140% and 152% of baseline, respectively). Mesenteric oxygen supply (mDO_2) and consumption (mVO_2) showed a significant reduction after burn to 60% and 54% of baseline, respectively. In contrast, animals treated with IL-1α showed an increase in postburn mDO_2 and mVO_2 to 124% and 128% of baseline, respectively. LPS-induced mesenteric hypoxia was also ameliorated by IL-1α treatment. Intestinal permeability, as assessed by the L/M ratio, showed a 7- and 10-fold elevation after thermal injury and LPS, respectively. In contrast, IL-1α-treated animals showed an increase of only 3- and 4-fold in the L/M ratio, respectively. Bacterial translocation was significantly increased in the burn/endotoxin group (7/7 vs. 1/7 in the sham group). IL-1α significantly reduced the rates of bacterial translocation (2/7).

Conclusions: IL-1α treatment attenuates mesenteric ischemia and reperfusion injury induced by thermal injury and endotoxemia, by improving mesenteric blood flow and oxygenation. Subsequently, IL-1α reduces intestinal permeability and bacterial translocation after burn and sepsis.

Introduction

The prognosis of extensively burned patients is dependent upon the presence of sepsis. The importance of ischemic damage and systemic inflammatory response syndrome (SIRS), initiated by mediators or cytokines in the pathogenesis of postburn multiple organ dysfunction syndrome (MODS), was demonstrated in serial clinical and experimental studies [1]. Sepsis syndrome also results from bacterial translocation (BT), in which gut bacteria and/or endotoxins (LPS) are thought to enter the portal bloodstream and/or lymph system [2, 3]. Circulating LPS was suggested to be the trigger for increased proinflammatory cytokine production, SIRS, and septic complications in injured patients [4]. The pathophysiological mechanism of sepsis is the increased release of inflammatory mediators and resulting imbalances between these substances and their antagonists. In cases of severe sepsis, the sequelae of the imbalance between inflammatory mediators and their antagonists can lead to endothelial injury, disseminated intravascular coagulation, and finally MODS [5]. Strategies against the occurrence of sepsis include hospital-wide infection control measures, modifying the immune system function, and minimizing the occurrence of BT [6].

Several mediators have been reported to be involved in the process of burn- and endotoxemia-induced ischemia and reperfusion injury to the intestine and BT [7]. There are some data available to implicate interleukin-1 (IL-1) as one of these mediators. IL-1 production by blood monocytes has been documented to be markedly decreased in severely burned patients [8]. These changes were more evident in patients complicated with organ injury, multiple organ failure, and systemic infection. The administration of interleukin-1α (IL-1α) was shown to improve survival in an animal model of burn wound sepsis [9]. This survival was associated with a decrease in positive blood cultures. IL-1 pretreatment was also shown to decrease ischemia and reperfusion injury [10]. Hence, we examined the ability of IL-1α to counteract intestinal ischemia and reperfusion injury and BT in a burn/sepsis chronic porcine model.

Methods

The experimental protocols were approved by the Animal Care and Use Committee of the University of Texas Medical Branch (ACUC # 90-09-103).

Surgical preparation

Studies were performed in 21 female mini-pigs (weight 20-25 kg). After an overnight fast, the pigs were sedated with intramuscular ketamine (10 mg/kg) and mechanically ventilated with 2% to 2.5% halothane after endotracheal intubation. A bilateral subcostal incision was performed. A transit time ultrasonic flow probe (6-8 mm, Transonic Systems Inc., Ithaca, NY) was placed on the superior mesenteric artery. A 6.5F catheter was positioned in the superior mesenteric vein. A Witzel jejunostomy was also performed using a 12F Foley catheter.

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. Through a neck incision a catheter was placed via the right common carotid artery into the abdominal aorta, and a Swan-Ganz thermal dilution catheter (Model 93 A-131-5F, American Edwards Laboratories, Anasco, PR) was positioned in the pulmonary artery through the right jugular vein. A 12F Foly catheter was inserted in the urinary bladder.

Experimental design

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

The pigs were randomized into three groups:

1) The burn/lipopolysaccharide (LPS) group (n=7) had a 40% total body surface area third-degree flame burn under general anesthesia as described above. The pigs were resuscitated according to the Parkland formula and received lactated Ringer's solution (4 mL/kg/percentage of total body surface area burned), starting immediately after the burn; half of which was given in the first 8 hours after burn and the remainder in the next 16 hours. Eighteen hours after burn, 100 μg/kg *Escherichia coli* LPS (0111:B4; Difco, Detroit, MI) was administered intravenously. During the second day of the experiment, burned animals received lactated Ringer's solution at 3,5 mL/m^2 burned area and 2 mL/kg/hr for daily maintenance.

2) The sham group (n=7) had a sham burn under anesthesia. Eighteen hours later, the animals received the diluent (0.9% NaCl) used for the endotoxin. Lactated Ringer's solution was administered at 2 mL/kg/hr for daily maintenance.

3) The treatment group (n=7) underwent the same procedure as the burn/LPS group, except for the administration of IL-1α (recombinant human IL-1α [lot IL-1 1/92], with specific activity of 8.8×10^8 units/mg, provided by Hoffmann-La Roche Inc., Nutley, NJ) intravenously at 1000 ng/kg, immediately after burn.

Mean arterial (MAP) and central venous (CVP) pressures were measured using transducers (P231D, Statham Gould, Oxnard, CA) connected to an Electronic Medicine Honeywell Recorder (Honeywell Inc., Pleasantville, NY) for electronic calculation of mean pressures. Cardiac output (CO) was determined by the thermal dilution technique using a Swan-Ganz catheter and a cardiac output computer (Model 9520, American Edwards Laboratories, Irvine, CA).

Superior mesenteric arterial (SMA) blood flow was measured with a transit time ultrasonic flow probe connected to a T101 ultrasonic meter (Transonic Systems Inc., Ithaca, NY).

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

Systemic vascular resistance index (SVRI) and mesenteric vascular resistance (MVR) were calculated with the following formulas:

Cardiac index ($L/min/m^2$) = cardiac output (L/min)/body surface area

SVRI (dyne · sec · cm^{-5} · m^2) = ([mean arterial pressure – central venous pressure] × 80)/cardiac index

MVR (dyne · sec · cm^{-5}) = ([mean arterial pressure – central venous pressure] × 80)/mesenteric arterial blood flow

Systemic oxygen delivery (DO_2), systemic oxygen consumption (VO_2), mesenteric oxygen delivery (mDO_2), and mesenteric oxygen consumption (mVO_2) were calculated as follows:

DO_2 (mL/min/m^2) = cardiac index × arterial oxygen content × 10

VO_2 (mL/min/m^2) = cardiac index × (arterial oxygen content – mixed venous oxygen content) × 10

mDO_2 (mL/min) = mesenteric arterial blood flow × arterial oxygen content/100

mVO_2 (mL/min) = mesenteric arterial blood flow × (arterial oxygen content – mesenteric oxygen content)/100

Arterial oxygen content (mL/dL) equals (Hb × 1.34) SaO_2 + (PaO_2 × 0.0031); mixed venous oxygen content (mL/dL) equals (Hb × 1.34) SvO_2 + (PvO_2 × 0.0031); mesenteric oxygen content (mL/dL) equals (Hb × 1.34) SmO_2 + (PmO_2 × 0.0031).

Permeability Assessment

After the animals had recovered from the surgical instrumentation, a solution of 10 grams lactulose and 5 grams mannitol, diluted in 60 mL distilled water (1,160 mOsm/kg), was given via the jejunostomy tube and urine was collected for a 6-hour period to obtain baseline measurements. Lactulose/mannitol (L/M) assessment was repeated every 6 hours. At the completion of collection, the urine was divided into aliquots and frozen at 20°C until assayed. Urinary lactulose and mannitol concentrations were simultaneously determined by the technique described by Fleming et al [11], using high pressure liquid chromatography coupled with pulsed amperometric detection (HPLC-PAD). Urine was 2-to 20-fold diluted with deionized water, depending on the collection volume. One milliliter of diluted urine was mixed with internal saccharide standards, desalted, vortex mixed, centrifuged, and filtered. Fifty microliter of the filtrate was injected onto a 250 x 40 mm anion exchange column (Dionex Carbopak, PAI, Houston, TX) and eluted with 0.15 mol/L NaOH, 1 mL/min at 20°C. Detection was by pulsed amperometric detection with a working gold electrode and silver/silver chloride reference electrode, with a detection potential of +0.05, oxidation potential of +0.06 and reduction potential of -0.95V. Quantification was by peak height analysis and peak height ratios, with internal standardization. This method offers excellent separation of the carbohydrates and precise detection at low concentrations (lactulose 0.3 mg/L). The amount of each sugar excreted in the urine during 6 hours was then converted to a

percentage of the amount of the given sugar, reflecting the excretion fraction of each sugar. By dividing the lactulose and mannitol excretion fractions, a permeability index - the L/M ratio- was calculated.

Testing for bacterial translocation

At the end of the 42 hours, the animals were anesthetized with 10 mg/kg intravenous ketamine and sacrificed with 5 mL intravenous saturated KCl. Using aseptic technique, through a midline laparotomy incision, peritoneal fluid and tissue samples from the proximal and distal mesenteric lymph nodes (MLNs), spleen, liver, kidney, lung, jejunum, ileum, cecum and colon were taken for bacteriologic cultures. Collected tissue samples were weighed and 0.5 g of each was homogenized in a tissue grinder with 4.5 mL nonbacteriostatic saline to create a 1:10 dilution of the original sample; 0.1 mL and 0.01 mL (of the 1:10 dilution) were inoculated onto a MacConkey agar plate and a Columbia Nutrient Agar (CNA) plate for isolation of gram-negative and gram-positive organisms, respectively. Therefore, one colony would represent $1x10^2$ and $1x10^3$ colony-forming units per gram of tissue, respectively, for each inoculum size. Limits of detection were 100 organisms per gram of tissue. Inoculated plates were incubated at 37°C for 24 and 48 hours and read with a Darkfield Quebec Colony Counter (Model 3330, American Optical Co., Buffalo, NY). Cultures were considered positive when more than 100 colonies per gram of tissue were found. All bacterial isolates were identified by biotype using a microscan 4 bacterial analyzer (Baxter, Sacramento, CA.)

Statistical analysis

The data are presented as mean ± SEM. Within-group analysis was performed by the analysis of variance (ANOVA) for repeated measurements with the Dunnett post hoc test. Between-groups analysis was performed by ANOVA for factorial analysis with the Bonferroni post hoc test. Bacteriologic tissue culture results were analyzed by the Fischer exact test. *P* values of <0.05 were considered statistically significant.

Results

Systemic hemodynamics:

Baseline hemodynamic measurements were similar in all groups. All animals survived the study period (Fig. 1 and Fig. 2).

After burn, MAP and CVP showed a slight elevation, but no significant differences were observed between groups. CO was elevated to 158% of baseline during the first 4 hours, returning to baseline 8 hours after burn. This increase was associated with a concomitant fall in SVRI to 65% of baseline level.

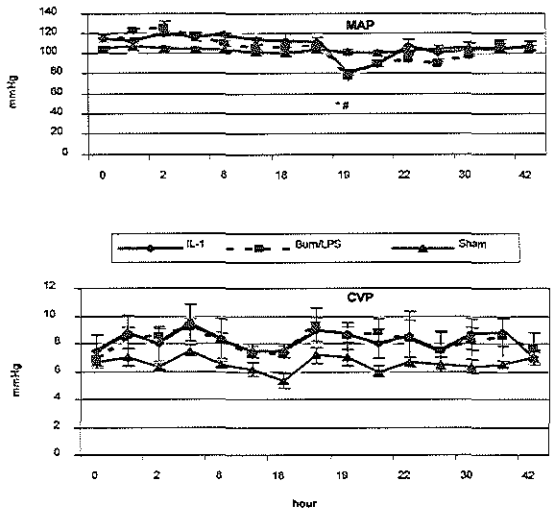

Figure 1. Mean arterial pressure (MAP) and central venous pressure (CVP) after burn (0 hour) and endotoxin (18 hours). IL-1α treatment had a marginal effect. P <0.05, * vs. baseline, # vs. sham, ¥ vs. IL-1α.

The second insult (administration of LPS) led to a typical biphasic response. During the early post-LPS phase, a decrease was noticed in MAP to 80% of baseline, and in CO to 67% of baseline. Four hours after LPS administration, all measurements were stabilized to baseline levels. A hyperdynamic period began to be manifest 8 hours after endotoxin infusion. Whereas CO showed a 20% increase, SVRI dropped to 64 % of baseline, at this time point. Throughout the study period, a slight elevation of the CVP

was shown in all burned animals. IL-1α treatment had a moderate impact on the hemodynamic alternations, seen after burn and LPS.

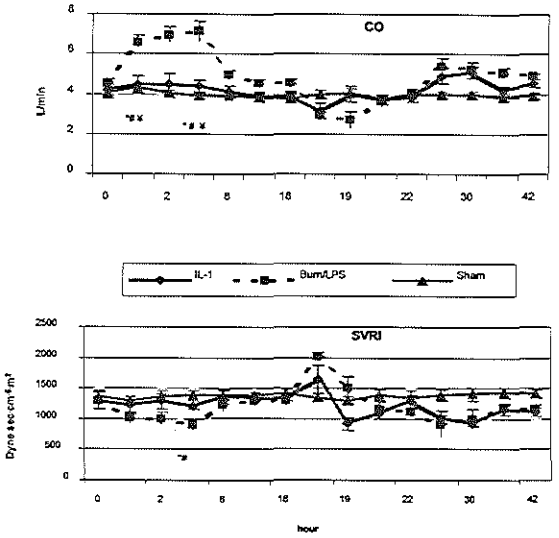

Figure 2. IL-1α ameliorated the changes in cardiac output (CO) and systemic vascular resistance index (SVRI) postburn (0 hour) and early after endotoxin (18 hours). $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. IL-1α.

Mesenteric hemodynamics:

Although thermal injury resulted in a hyperdynamic status with an increased CO, SMA blood flow decreased significantly to approximately 59% of baseline level during the first 2 hours after burn (Fig. 3). In contrast to the previously observed postburn reduction in systemic vascular resistance, MVR showed a significant increase (195% of baseline) during the early postburn phase (Fig. 4). During the late postburn phase, starting 4 hours after insult, a mesenteric reperfusion phase became manifested, as SMA blood flow increased by 45% over baseline. Compared to burned animals not receiving IL-1α treatment, IL-1α–treated animals showed no reduction in SMA blood flow following thermal insult. On the contrary, SMA blood flow in this group showed an increase of 40% of baseline (Fig. 3). Animals in the IL-1α treatment group maintained, in contrast to nontreated burned animals, a stable MVR near baseline during this early mesenteric vasoconstrictive phase (Fig. 4).

At 18 hours postburn, mesenteric hemodynamic measurements were comparable to baseline levels in all groups.

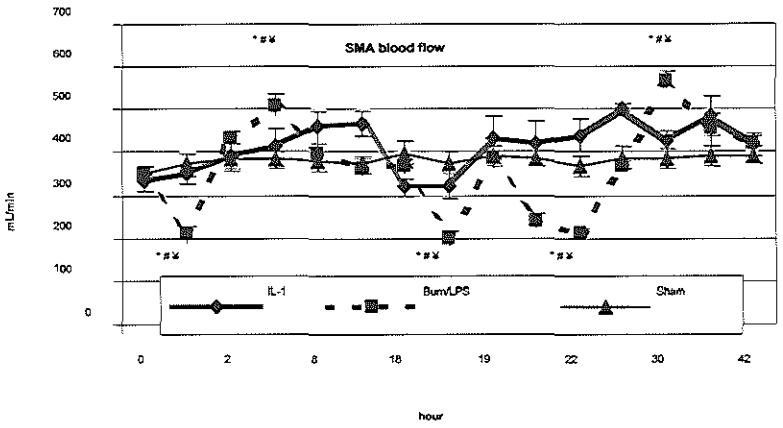

Figure 3. Superior mesenteric artery (SMA) blood flow was significantly reduced and showed a pattern of ischemia and reperfusion after burn (0 hour) and endotoxin (18 hours). IL-1α administration significantly improved SMA blood flow. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. IL-1α.

Administration of LPS to burned animals resulted in a biphasic response of mesenteric ischemia and reperfusion. The second insult yielded a significant mesenteric vasoconstriction with an increase of MVR to 158% of baseline during the first 8 hours post-LPS.

Similarly, SMA blood flow decreased significantly to 60% of baseline during the same time of maximum increase of MVR. Burned animals treated with IL-1α showed no signs of mesenteric vasoconstriction after the second insult (LPS). Compared to nontreated animals, IL-1α treatment resulted in a significant improvement of SMA blood flow after the second impact (LPS). Eight hours post-LPS, SMA blood flow reached a value of 152% of baseline in the IL-1α group, whereas MVR was significantly reduced to 56% of baseline (Fig. 3 and Fig. 4).

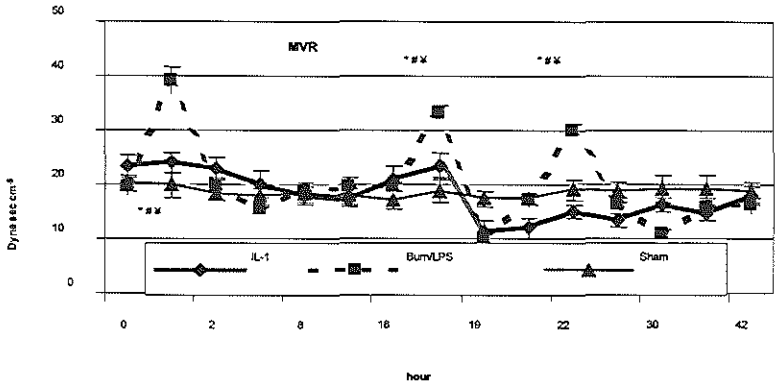

Figure 4. Alterations in mesenteric vascular resistance (MVR) after burn (0 hour) and endotoxin (18 hours). IL-1α had a positive impact on MVR. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. IL-1α.

Systemic oxygen delivery and consumption:

During the first 4 hours after burn, the burn/LPS group showed a significant increase in both DO_2 (150% of baseline) and VO_2 (204% of baseline). The IL-1α group showed moderate changes in DO_2 and VO_2 after burn, with an increase to 113% and 126% of baseline, respectively (Fig. 5).

One hour after LPS administration, a significant drop in DO_2 was noticed in the burn group (57% of baseline). VO_2 was reduced to 68% of baseline during this early post-LPS phase. During the post-LPS hyperdynamic phase, DO_2 and VO_2 showed no significant alterations. Animals treated with IL-1α showed some changes post-LPS, but of no significance (Fig. 5).

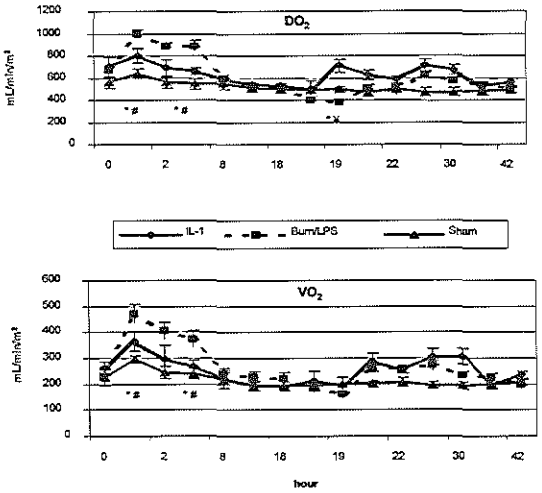

Figure 5. Administration of IL-1α slightly affected systemic oxygen delivery (DO_2) and oxygen consumption (VO_2) after burn (0 hour) and endotoxin (18 hours). P <0.05, * vs. baseline, # vs. sham, ¥ vs. IL-1α.

Mesenteric oxygen delivery and consumption:

In the early phase after burn, mDO_2 and mVO_2 showed a significant fall to 60% and 54% of baseline levels, respectively. On the contrary, animals treated with IL-1α showed an increase in postburn mDO_2 and mVO_2 to 124% and 128% of baseline, respectively (Fig. 6).

Administration of LPS resulted in a significant reduction in mDO_2 to 50% of baseline. Postburn treatment with IL-1α abrogated this deleterious impact of LPS and mDO_2 showed an increase to 121% of baseline. Correspondingly, post-LPS diminished mVO_2 was attenuated in animals receiving IL-1α (Fig. 6, 79% vs. 136% of baseline).

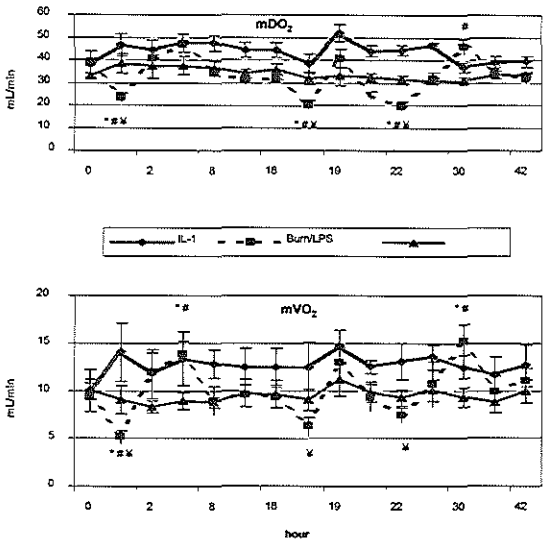

Figure 6. burn (0 hour) and endotoxin (18 hours) significantly reduced mesenteric oxygen supply (mDO_2) and oxygen consumption (mVO_2). These detrimental effects were attenuated by IL-1α treatment. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. IL-1α.

Lactulose and mannitol assay

No differences were found between groups in baseline measurements of the L/M ratio. The L/M ratio for burned animals began to increase 6 hours after burn, reaching a 7-fold elevation 12 hours after burn in the burn/LPS group, compared with only a 3-fold increase in the treatment group. The differences between groups were significant at this time point.

LPS administration to burned animals caused a clear deterioration in the mucosal permeability, as assesed by the L/M ratio. The L/M excretion ratio was 10- and 8-fold increased in the burn/LPS group at 12 and 18 hours after LPS administration, respectively. On the contrary, animals treated with IL-1α showed only a 4- and 3-fold increase at these time points. Differences between groups were significant (Fig. 7).

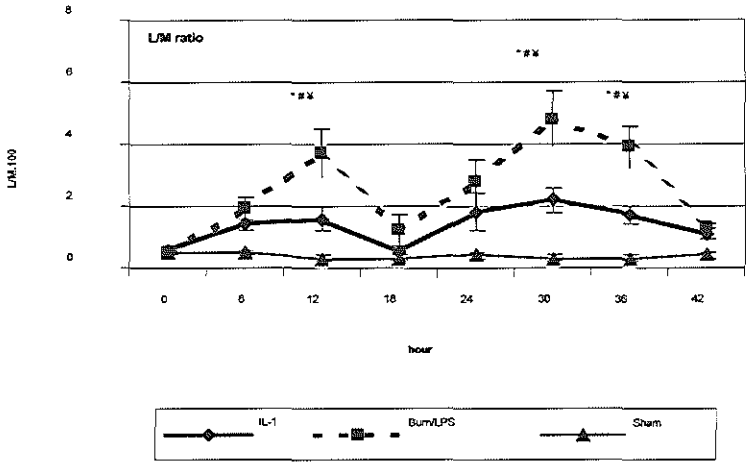

Figure 7. Intestinal permeability, as assessed by the lactulose/mannitol (L/M) excretion ratio, was significantly increased after burn (0 hour) and endotoxin (18 hours). IL-1α administration significantly reduced intestinal permeability. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. IL-1α.

Quantitative bacteriologic culture of tissue samples:

Tissue cultures were tested positive for enteric bacteria in all animals in the burn/LPS group. In contrast, only 1 out of 7 animals in the sham group showed positive tissue cultures ($P < 0.05$). IL-1α treatment yielded a significant reduction in the rates of positive tissue cultures, whereas only 2 out of 7 animals in this group tested positive ($P < 0.05$ vs. burn/LPS).

Only tissue cultures with enteric bacteria of the same biotype as that found in the intestine of corresponding animal were interpreted as evidence of bacterial translocation (Table 1).

In the burn/LPS group, 100% of the animals showed bacterial translocation to the MLNs and liver, 86% to the spleen and lung, and 57% to the kidney (Fig. 8). On the contrary, only 28% of the treated animals showed bacterial translocation to the MLNs and spleen ($P<0.05$), 14% to the liver and lung ($P<0.05$), and none to the kidney (Fig. 8).

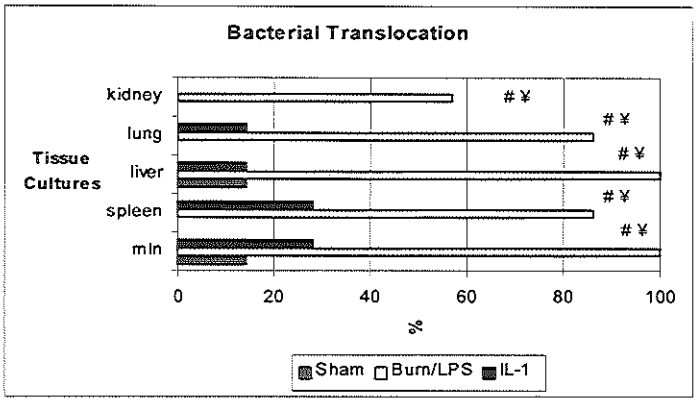

Figure 8. Incidence of bacterial translocation to remote organs was significantly increased in the burn and endotoxin group. IL-1α treatment yielded a significant reduction in the rates of positive tissue cultures with enteric bacteria. Data are presented as percentages of harvested tissue samples. $P < 0.05$, # vs. sham, ¥ vs. IL-1α.

Table 1. Rates, types and origins of tissue isolates from the three study groups. MLN= mesenteric lymph nodes, j= jejunum, i=ileum, c=cecum.

tissue samples	positive/total	organism	origin
		Sham	
MLN	(1/7)	Escherichia coli	i/c
		Klebsiella pneumoniae	i/c
		Enterococcus faecium	j/c
liver	(1/7)	Klebsiella pneumoniae	i/c
		Burn/LPS	
MLN	(7/7)	Streptococcus bovis	j/i/c
		Staphylococcus hyicus hyicus	j/i/c
		Enterococcus fecalis	j/i/c
		Klebsiella pneumoniae	j/i/c
Spleen	(6/7)	Staphylococcus hyicus hyicus	j/i/c
		Enterococcus fecalis	j/i/c
		Escherichia coli	j/i/c
Liver	(7/7)	Staphylococcus hyicus hyicus	j/i
		Escherichia coli	j/i/c
		Enterococcus fecalis	j/i/c
		Klebsiella pneumoniae	j/i/c
Lung	(6/7)	Staphylococcus hyicus hyicus	j/i/c
		Enterococcus fecalis	j/i/c
		Enterococcus faecium	j/i/c
		Streptococcus bovis	j/i/c
		Klebsiella pneumoniae	j/i/c
Kidney	(4/7)	Staphylococcus aureus	i/c
		Escherichia coli	j/i/c
		Stafhylococcus hyicus hyicus	c
		IL-1α	
MLN	(2/7)	Enterococcus fecalis	i/c
		Klebsiella pneumoniae	i/c
		Escherichia coli	i/c
Spleen	(2/7)	Escherichia coli	i/c
Liver	(1/7)	Escherichia coli	i/c
		Enterococcus fecalis	i/c
Lung	(1/7)	Enterococcus fecalis	i/c
Kidney	(0/7)		

Discussion

The immense inflammatory focus incited by the burn causes the release of numerous cytokines and inflammatory mediators that have many systemic effects, and may ultimately result in multiple organ dysfunction. Recently, the role of the gut as a cytokine-generating organ in the post-injury inflammatory response has been studied [12]. As the causal relation of bacterial translocation or gut-derived cytokines to the development of sepsis and multiple organ dysfunction syndrome (MODS) has become more apparent and more complex, the need to identify the mechanisms involved in the pathophysiology of post-injury gut dysfunction is increasing [13]. Therefore, we recently reported on possible mediators in this complex process [7]. Among the molecular mediators relevant to the pathogenesis of burn injury-associated changes, cytokines have attracted special attention. IL-1 is presumed to be a key component of the inflammatory mediator cascade, directing the host response to infection, injury or inflammation [14]. Since IL-1α has multiple effects on the elements of the immune system, we examined in this study the ability of IL-1α to reduce bacterial translocation in a porcine model of burn and endotoxemia. Although the potential role of bacteria/endotoxin translocation and its clinical relevance remains controversial, many lines of evidence support the concept that early gut hypoperfusion sets the stage for progressive gut dysfunction, such that the gut becomes a reservoir for pathogens and toxins that contribute to systemic inflammatory response syndrome (SIRS) and MODS [15]. The consequences of bacterial translocation for the host were not the issue addressed in this study, but rather the pathophysiology of this process. Our animal model is based on the two hits concept [16, 17], referring to the episodes of endotoxemia to which burned patients are frequently exposed [18]. IL-1α was administered as a single dose (1000 ng/kg) directly after the onset of a major thermal injury in order to evaluate the action of IL-1α as a treatment modality. The efficacy of this treatment scheme in improving survival in a burn and sepsis animal model has been documented in a previous study [9].

The results of this experiment clearly show that postburn administration of IL-1α significantly reduces the occurrence of bacterial translocation. In contrast to an occurrence rate of 100% in animals with combined burn and LPS insults, bacterial

translocation was observed in only 28% of animals receiving burn and LPS and treated with IL-1α. These findings suggest several potential explanations. It is possible that IL-1α has enhanced bacterial clearance capacities in treated animals. Also, IL-1α treatment may have altered local or systemic factors involved in the pathophysiological pathways of the bacterial translocation process. There is enough evidence in the literature to support the first theory. IL-1α has been reported to decrease rates of positive blood cultures and increase the absolute neutrophil counts in a murine model of burn wound sepsis [9]. In addition, IL-1α could have increased the animal's ability to kill translocated bacteria via its influence on hematopoietic growth factors known to enhance the proliferation and functional capacity of neutrophils and macrophages [19].

The second hypothesis regarding the possible impact of IL-1α on potential initiators of bacterial translocation was the subject of our study. A direct or indirect action of IL-1α on cellular level could have interacted with the intestinal mucosal integrity, leading to decrease translocation of enteric bacteria. To examine whether IL-1α interferes with intestinal permeability, we measured the L/M excretion ratio. The clinical relevancy of this assessment method has been shown in previous studies [20, 21], in which an association between increased L/M ratio and the occurrence of postburn infections was observed in humans. Furthermore, an increased L/M ratio was found to correlate with oxidant-induced damage and the occurrence of bacterial translocation in our combined burn and sepsis model [22]. Burn yielded in our study a 7-fold increase in intestinal permeability during the first 12 hours after injury, as indicated by the L/M ratio. This detrimental impact of thermal trauma on intestinal permeability was augmented after the administration of endotoxin. During this post-injury toxemic phase the L/M ratio was 10-fold elevated during the first 12 hours after LPS infusion. Eighteen hours post-LPS, intestinal permeability was still 8-fold increased. These results confirm the data of previous investigators (including ourselves) [23, 22]. Our data clearly show that postburn administration of IL-1α can counteract the negative effects of both challenges, i.e. the initial burn injury and the secondary endotoxemia. Intestinal permeability in animals treated with IL-1α was significantly reduced to 3- and 4-fold after burn and endotoxin, respectively. The beneficial effect of IL-1α on intestinal permeability might

be the result of a direct pathway by which IL-1α protects the intestinal integrity. IL-1 has been shown to increase intestinal crypt cell mitoses and intestinal villus height, leading to improving intestinal cytoarchitecture and reducing bacterial translocation after a 32% total body surface area burn in mice [24]. A similar effect could explain the impact of IL-1α in our study, however we have not explored this possibility in the current study by means of histological studies of intestinal samples. The hypothesis that IL-1α indirectly influences the intestinal permeability by interacting with other factors or pathways, such as mucosal perfusion, was the subject of this study.

Ischemia and reperfusion injury to the intestinal mucosa, caused by burn and endotoxin, and its detrimental impact on intestinal permeability and bacterial translocation have been profoundly illustrated in previous studies from our lab [7, 22]. The data of the current study confirm our previous findings of the occurrence of mesenteric ischemia and reperfusion injury following burn and endotoxin, despite the presence of adequate resuscitation status. The observation of a significant mesenteric vasoconstriction (decreased SMA blood flow and increased mesenteric vascular resistance) during the early phase after thermal injury, while the animals were showing a hyperdynamic status (increased cardiac output and decreased systemic vascular resistance), demonstrates the selective negative impact of thermal injuries on mesenteric circulation. Administration of IL-1α directly postburn led to moderate alterations in the systemic hemodynamic parameters. In contrast, a significant improvement of the mesenteric blood supply was noticed after IL-1α postburn treatment. This beneficial effect of IL-1α treatment on intestinal blood supply in burned animals could be explained by an interaction of IL-1α with vasodilators, such as some of the prostanoids. IL-1 has been documented to cause an alteration in prostaglandin concentrations, increasing prostaglandin E (PGE_2) and prostacyclin (PGI_2) [14]. In another study IL-1α was found to act directly on endothelial cells, inducing the production of vasodilators such as PGI_2 [25]. A similar impact of IL-1α on systemic and mesenteric circulations was observed during the postburn septic phase, i.e. after administration of LPS. IL-1α was found to significantly reduce mesenteric vascular resistance (MVR) and improve SMA blood

flow. The effect on systemic hemodynamic parameters was only noticeable during the first 4 hours after LPS infusion.

Although systemic oxygen delivery (DO_2) and consumption (VO_2) were increased following thermal injury, mesenteric oxygen supply (mDO_2) was significantly reduced early postburn. The discrepancy between the changes measured in both systemic and mesenteric oxygenation status after burn, demonstrates the independence of the impact of thermal injury on the mesenteric oxygenation status, as previously seen [22]. During the same period, mVO_2 became mDO_2-dependent, with a significant reduction, indicating the inability of the gut to compensate inadequate oxygen delivery by increasing oxygen extraction, resulting in tissue hypoxia. Early hypoxia in the splanchnic region has been implicated in the process of developing secondary organ failure after major injuries or sepsis [26]. Postburn administration of IL-1α resulted in a significant improvement in mDO_2. Mesenteric hypoxia, as indicated by decreased mVO_2, was not noticed in animals in the treatment group. The enhancement in oxygen supply to meet the increased oxygen demand was only observed in the mesenteric circulation, and no significant differences were found between treated and untreated burned animals with respect to DO_2 or VO_2. During the postburn septic phase, a typical biphasic response was observed in mDO_2 and mVO_2, with a more pronounced flow-dependent mesenteric hypoxic period lasting for approximately 8 hours. On the contrary, LPS had only moderate effects on DO_2 and VO_2. LPS alone has been shown to cause mesenteric hypoperfusion, yielding intestinal mucosal acidosis and increased permeability [27]. After the second insult (LPS), burned animals receiving IL-1α showed a significant improvement in their mesenteric oxygenation status compared with nontreated animals.

The data of this study clearly show that postburn administration of IL-1α attenuates the mesenteric ischemia and reperfusion injury induced by thermal injury and postburn endotoxemia. Our data are in correspondence with other data collected from other studies, documenting the beneficial effects of IL-1α in other organs against ischemia and reperfusion injury. The results of those studies have indicated that low doses of IL-1α can be used as a therapeutic agent to precondition the heart against ischemia and reperfusion injury [28] and to decrease the onset of myocardial ischemia and

reperfusion injury [10]. However, this particular effect of IL-1α seems to be of a selective nature and is probably organ-specific, as IL-1 was found to be unlikely beneficial in the recovery of renal function after ischemia [29]. The mechanisms by which IL-1 interacts with other mediators and modulators in the cascade of ischemia and reperfusion injury induced by burn and sepsis and the consequential effects of such actions on different organs, have not yet been fully elucidated. Therefore, further studies on this particular field are warranted.

Conclusion

The cytokine IL-1α appears to play an important role in intestinal perfusion and oxygenation after burn and sepsis. Postburn treatment with IL-1α reduces intestinal permeability and bacterial translocation, most likely by attenuating mesenteric ischemia and reperfusion injury.

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CHAPTER 7

Hepatic Blood Flow and Oxygen Consumption after Burn and Sepsis

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Abstract

Background: Alteration in the hepatic circulation after burn and in sepsis seems to be an essential component in the development of multiple organ failure.

Methods: Female pigs (n=12, 20-25 kg) were instrumented 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 6 of them received 40% total body surface area third-degree burn. A total of 100 μg/kg *Escherichia coli* LPS was intravenously administered at 18 hours after burn. All animals were studied for 42 hours.

Results: Thermal injury resulted in a 48% decrease in hepatic arterial blood flow despite maintenance of normal cardiac output, resulting in a fall in hepatic oxygen delivery rate. Portal venous blood flow showed a 32% increase at 4 hours after burn. Post-LPS portal blood flow was significantly reduced for a period of 8 hours (51% of baseline, $P<0.05$, ANOVA). The hepatic arterial blood supply was also significantly reduced (12-67% of baseline, $P<0.05$, ANOVA) during the first 4 hours after LPS, indicating loss of the hepatic arterial response. The following 12 hours, a hepatic reperfusion phase was observed with an elevation of the hepatic arterial blood flow to 152% of baseline ($P<0.05$, ANOVA). Postburn endotoxemia resulted in a significant decrease of hepatic oxygen delivery (88%) and hepatic oxygen consumption (79%). Although the burn injury did not affect the portal venous pressure, postburn endotoxemia caused a significant portal hypertension during a period of 8 hours (225% of baseline, $P<0.05$, ANOVA).

Conclusions: Postburn sepsis amplifies the selective vasconstrictive impact of thermal injury on hepatic arterial blood flow, yielding a pronounced ischemia and reperfusion injury, associated with a critical reduction of hepatic oxygen delivery and consumption. A postburn septic challenge induces portal hypertension, which may account for previously documented gut barrier dysfunction.

Introduction

Sepsis and multiple organ dysfunction syndrome (MODS) remain to be the most common causes of the high morbidity and mortality rates after major burn injuries [1-3]. It has been postulated that the amplified reaction of the primed inflammatory response system of thermally injured patients to a subsequent insult, initiated by bacteria and their by-products (endotoxins), is responsible for the typical pathophysiological alterations seen in post-injury sepsis [2]. Although a clear defined pathophysiologic mechanism has not yet been defined, the gastrointestinal tract has been postulated to be a major contributor to postburn sepsis [4]. The intestinal mucosal barrier normally prevents the enteric bacteria and their by-products from escaping and reaching extraintestinal organs. However, under certain circumstances, the integrity of intestinal mucosa seems to be disrupted, allowing an exaggerated leaking of endogenous bacteria or endotoxins to the portal circulation; this is a process called bacterial translocation [5,6].

As the hepatic mononuclear phagocytic system (Kupffer cells) seems to play a pivotal role in the clearance of translocating bacteria, endotoxin, or both, from the portal circulation [7], 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.

Phagocytosis of bacteria and sequestering of endotoxin by hepatic Kupffer cells impose a high metabolic demand on the mononuclear phagocytic system [8]. Intermediary metabolism and energy production have an absolute dependence on oxygen. Because oxygen can not be stored intracellularly, inadequate oxygen availability rapidly leads to cellular dysfunction and ultimately cell death with the net result being organ failure. Hence, the importance of the hepatic perfusion for the performance of Kupffer cells.

The present study was designed to define the hemodynamic response and the tissue perfusion of the liver to severe thermal injuries and postburn endotoxemia in a burn/endotoxin porcine model.

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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 (ACUC # 90-09-103).

Twelve female mini-pigs, each weighing between 20 and 25 kg, were prepared surgically 5 days before the experiment. After an overnight fast, the pigs were sedated with intramuscular ketamine (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. The 6.5 F catheters were positioned in the superior mesenteric vein and the left hepatic vein. Witzel jejunostomy was also performed using a 12 F Foley catheter. The abdomen was closed in layers.

After surgery, the animals were kept in special 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, PR) was positioned in the pulmonary artery via the right jugular vein. A 12 F Foly catheter was inserted in the urinary bladder.

The animals were kept in special slings for monitoring. All animals tolerated the slings well, without any signs of stress or need for sedation. Throughout the study, all animals received enteral feeding (Osmolite) at 25 mL/hr and nothing per os. Baseline data were collected after complete recovery from anesthesia.

The pigs were randomized into 2 groups:

1) The burn/LPS group (n=6) had a 40% total body surface area (TBSA) third-degree flame burn under general anesthesia as described above. The pigs were resuscitated according to the Parkland formula and received lactated Ringer's solution, 4 mL/kg/%TBSA burn, starting immediately after burn. Half of the fluid was given in the first 8 hours after burn and the remainder in the following 16 hours. Eighteen hours after burn, 100 μg/kg *E. coli* lipopolysaccharide (LPS) (0111:B4; Difco, Detroit, MI) was intravenously administered. During the second day of the experiment, burned animals

received lactated Ringer's solution at 3,5 mL/m^2 burned area and 2 mL/kg/hr for daily maintenance.

2) The sham group (n=6) had a sham burn under anesthesia. Eighteen hours later the animals received the diluent (0.9 % NaCl) used for the endotoxin. Lactated Ringer's solution was administered at 2 mL/kg/hr for daily maintenance.

Mean arterial (MAP), central venous (CVP), and portal venous (PVP) pressures were measured using transducers (P231D, Statham Gould, Oxnard, CA) that were connected to an Electronic Medicine Honeywell Recorder (Honeywell Inc., Pleasantville, NY) for electronic calculation of mean pressures. Cardiac output (CO) was determined by the thermal dilution technique using a Swan-Ganz catheter and a cardiac output computer (Model 9520, American Edwards Laboratories, Irvine, CA).

Hepatic arterial blood flow (Qh) and portal venous blood flow (Qp) were measured with transit time ultrasonic flow probes connected to a T101 ultrasonic meter (Transonic Systems Inc., Ithaca, NY).

Systemic and hepatic hemodynamics were measured and blood samples were drawn for determination of arterial, mixed venous, and hepatic blood gases at baseline and 14 consecutive time points, starting 1 hour after burn.

Systemic vascular resistance index (SVRI), hepatic arterial vascular resistance (HAVR), and hepatic portal vascular resistance (HPVR) were calculated with the following formulas:

SVRI (dyne·sec·cm^{-5}·m^2)=[(MAP - CVP) x 80] / CI

HAVR (dyne·sec·cm^{-5})=[(MAP - CVP) x 80] / Qh

HPVR (dyne·sec·cm^{-5})=[(PVP - CVP) x 80] / Qp

Systemic O_2 delivery (DO_2), systemic O_2 consumption (VO_2), hepatic O_2 delivery (hDO_2), and hepatic O_2 consumption (hVO_2) were calculated with the following formulas:

DO_2=CI x CaO_2 x 10 (mL/min/m^2)

VO_2=CI x (CaO_2 - CvO_2) x 10 (mL/min/m^2)

hDO_2 =Qh x CaO_2 / 100 (mL/min)

hVO_2 =Qh x (CaO_2 - ChO_2) / 100 (mL/min)

Where CI = cardiac index ($L/min/m^2$), CaO_2 (arterial oxygen content, mL/dL) = (Hb x 1.34) SaO_2 + (PaO_2 x 0.0031), CvO_2 (mixed venous oxygen content, mL/dL) = (Hb x 1.34) SvO_2 + (PvO_2 x 0.0031), and ChO_2 (hepatic oxygen content, mL/dL) = (Hb x 1.34) ShO_2 + (PhO_2 x 0.0031).

At the end of the 42 hours, the animals were anesthetized with 10 mg/kg intravenous ketamine and killed with 5 mL intravenous saturated KCl.

The data are presented as mean ± SEM. Within-group analysis was performed by the analysis of variance (ANOVA) for repeated measurements with the Dunnet post hoc test. Between-groups analysis was performed by ANOVA for factorial analysis with the Bonferroni post hoc test. All *P* values of <0.05 were considered statistically significant.

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 (Figs. 1 and 2) hemodynamics within baseline range.

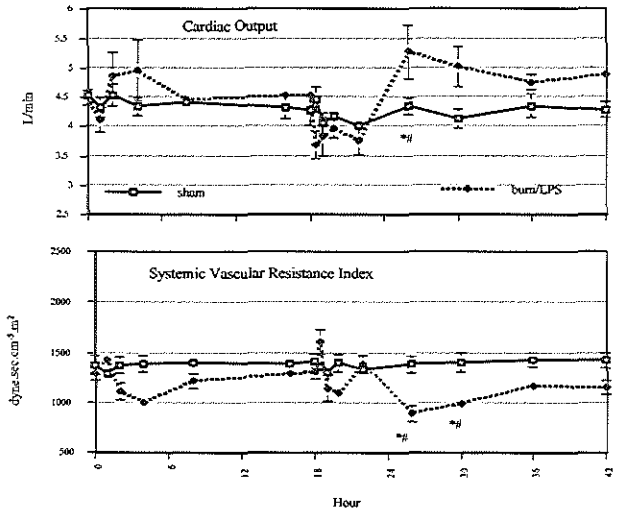

Figure 1. Cardiac output (CO) and systemic vascular resistance index (SVRI) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). $P < 0.05$, * vs. baseline, # vs. sham.

After thermal injury, cardiac output (CO) showed a slight increase during the first 6 hours, returning to baseline 8 hours postburn (Fig. 1). This increase was associated with a concomitant fall in the systemic vascular resistance, whereas the systemic vascular resistance index (SVRI) decreased to 78% of baseline level (Fig. 1). There were no significant differences in mean arterial pressure (MAP), central venous pressure (CVP), serial hematocrits (Hct), or urine production (UP) between the 2 groups (Figs. 2 and 3).

After 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 post-endotoxin (Fig. 1). At this time point, SVRI dropped to 69% of baseline (Fig. 1). In

the burn/LPS group, MAP showed a 14% decrease immediately after LPS infusion (Fig. 2). During the further post-LPS course, no significant differences were noticed between groups in MAP, CVP, Hct, or UP (Figs. 2 and 3)

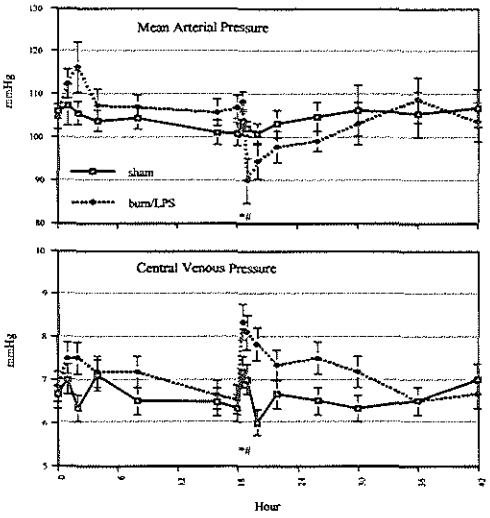

Figure 2. Mean arterial pressure (MAP) and central venous pressure (CVP) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). $P < 0.05$, * vs. baseline, # vs. sham.

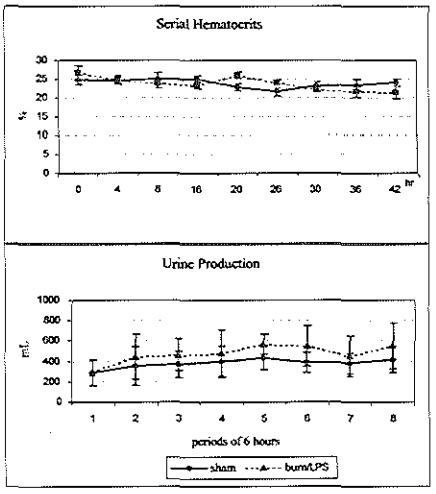

Figure 3. Serial hematocrits (Hct) and urine production (UP) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). No statistically significant differences were measured.

Hepatic Hemodynamics

Hepatic Arterial Circulation

In contrast to the CO, which remained within baseline range, Qh decreased significantly to approximately 52% of baseline level during the first 4 hours postburn (Fig. 4). This fall in Qh was associated with a significant increase in hepatic arterial vascular resistance (HAVR), reaching 462% of baseline as early as 1 hour postburn (Fig. 5).

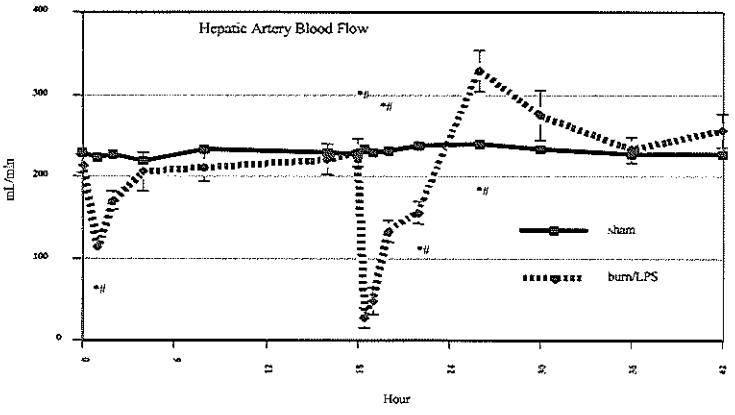

Figure 4. Hepatic arterial blood flow (Qh) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). $P < 0.05$, * vs. baseline, # vs. sham.

Hepatic arterial hemodynamic measurements recovered completely in all animals to baseline values at 18 hours after burn (Figs. 4 and 5).

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 16-fold increase of HAVR during the first hour after LPS (Fig. 5). HAVR remained significantly increased (550% of baseline) for a period of 6 hours after LPS administration (Fig. 5). Correspondingly, Qh decreased significantly to 12% of baseline during the same time of maximum increase of HAVR (Fig. 4). After an

initial recovery of Qh to baseline values 6 hours after LPS, a marked elevation (127-152% of baseline) was noticed during the following 6 hours (Fig. 4).

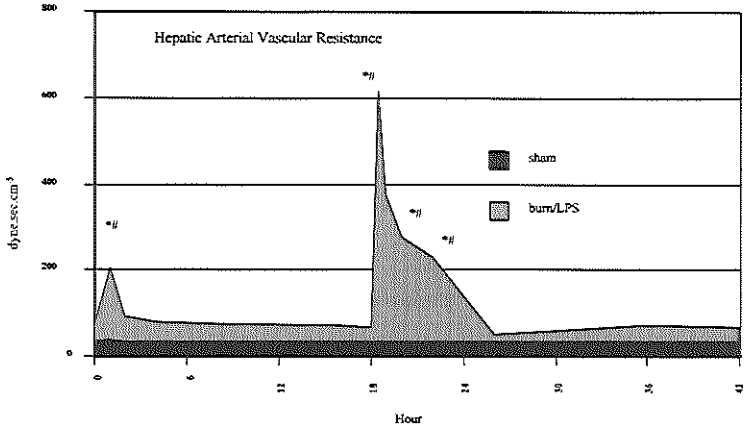

Figure 5. Hepatic arterial vascular resistance (HAVR) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). $P < 0.05$, * vs. baseline, # vs. sham.

Hepatic Portal Circulation

During the first 4 hours after burn, Qp increased to approximately 132% of baseline (Fig. 6). Although no important changes were observed in the pattern of measured PVP, HPVR showed a 34% decrease of baseline values during the same time period (Figs. 7 and 8).

Similar to the hepatic hemodynamic arterial variables, portal hemodynamic measurements showed the same pattern of recovery to baseline values at 18 hours after burn in all animals (Figs. 6, 7 and 8).

The second insult yielded significant alterations in the portal circulation, lasting for a prolonged time period. The HPVR showed a 2- to 4-fold increase during the first 8 hours after LPS administration (Fig. 7). During this early septic phase, a significant portal hypertension was noticed, whereas measured PVP was elevated to approximately 225% of baseline values (Fig. 8). Qp showed a biphasic response after LPS administration.

During the first 8 hours after LPS, Qp decreased to approximately 51% of baseline (Fig. 6). After a transient recovery to baseline, a hyperdynamic phase with an elevation of Qp to 147% of baseline began at 30 hours (12 hours after LPS) and remained till the end of the study period (Fig. 6). During this late septic period, HPVR was decreased to 63% of baseline and PVP was slightly increased to 121% of baseline (Figs. 7 and 8).

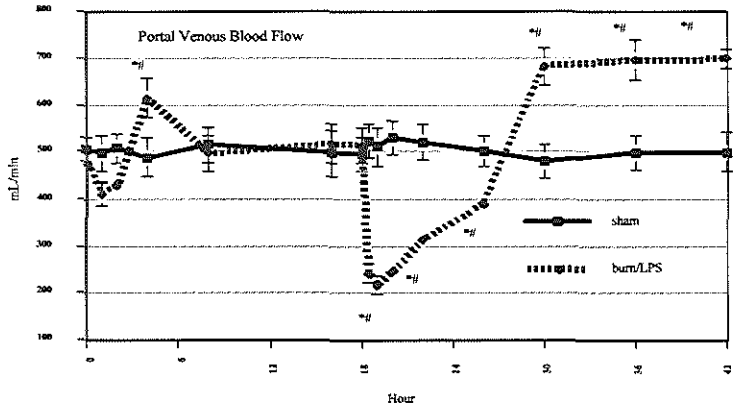

Figure 6. Portal venous blood flow (Qp) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). $P < 0.05$, * vs. baseline, # vs. sham.

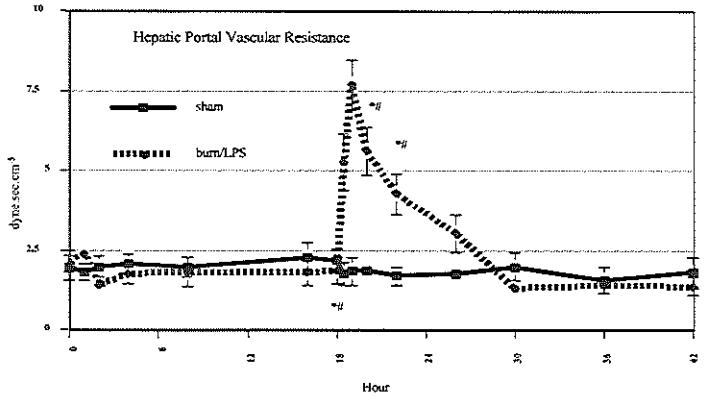

Figure 7. Hepatic portal vascular resistance (HPVR) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). $P < 0.05$, * vs. baseline, # vs. sham.

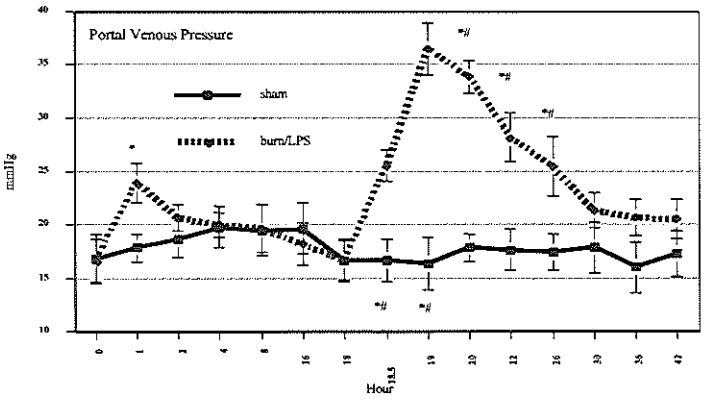

Figure 8. Portal venous pressure (PVP) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). $P < 0.05$, * vs. baseline, # vs. sham.

Systemic oxygen delivery and consumption

After a transient reduction, DO_2 and VO_2 showed a marked increase during the first 6 hours after burn (Fig. 9).

Administration of LPS resulted in a significant drop in DO_2 during the first hour. VO_2 was moderately decreased at this time point (Fig. 9). During the post-LPS hyperdynamic phase, both DO_2 and VO_2 were increased.

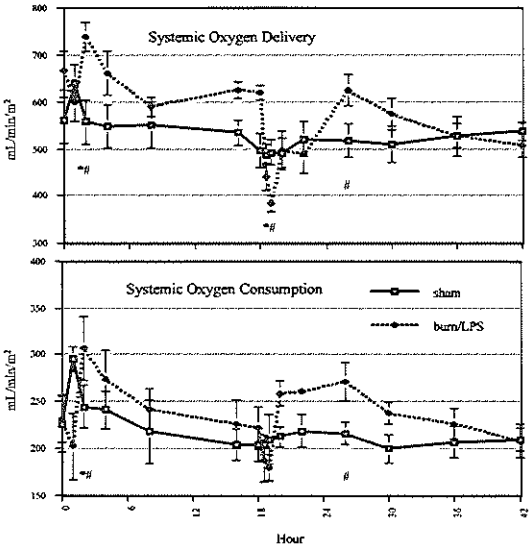

Figure 9. Systemic oxygen delivery (DO_2) and oxygen consumption (VO_2) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). P <0.05, * vs. baseline, # vs. sham.

Hepatic oxygen delivery and consumption

hDO_2 was significantly reduced to 63% of baseline during the first 2 hours after burn. In contrast, hVO_2 was sustained within baseline values with a slight decrease of 16%, implicating increased hepatic oxygen extraction capacities during the postburn phase (Fig. 10).

As a result of the second insult (LPS administration), hDO_2 decreased significantly to 12% of baseline during the first hour after LPS and remained as low as 56% of baseline at 4 hours after LPS administration (Fig. 10). hVO_2 showed a similar pattern, whereas a significant decrease of 21% to 65% of baseline values was calculated

during the first 4 hours after LPS (Fig. 10). After a transient increase in both oxygen delivery and consumption rates at 6 hours after LPS, a recovery to baseline range was reached at 36 hours (18 hours after LPS).

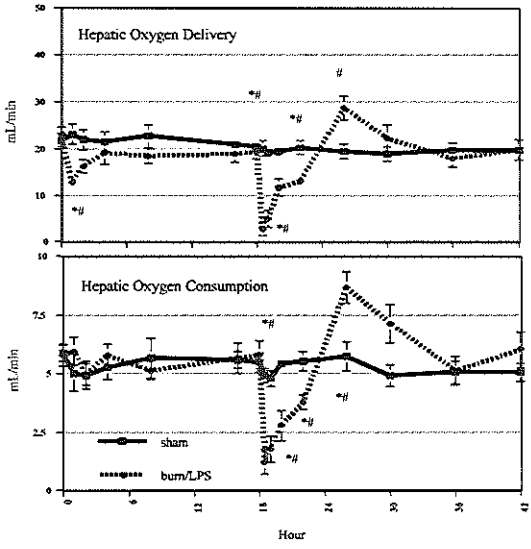

Figure 10. Hepatic oxygen delivery (hDO_2) and hepatic oxygen consumption (hVO_2) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours after burn). $P < 0.05$, * vs. baseline, # vs. sham.

Discussion

Burn injuries have been documented to have a negative impact on hepatic perfusion. The influence of severe thermal injury on hepatic blood flow was investigated in a 50% TBSA burn rat model, by using the tricarbocyanine dye indocyanine green (ICG) [9]. Hepatic blood flow was decreased significantly by 0.5 hour after burn and remained approximately 20% below normal for 24 hours. The intrinsic efficiency of the liver in removing the ICG dye from the systemic circulation was also reduced.

In an ovine model, the effect of 40% TBSA third-degree burn on effective liver blood flow was determined, by using the galactose infusion technique [10]. The effective liver blood flow was decreased by 50% in the first 5 hours after burn, even when the animals were resuscitated to baseline cardiac output values.

In our model, Qh showed a transient but significant decrease (48% of baseline) shortly after burn, which was associated with a 4.6-fold increase in HAVR. The measuring of a moderate increase in CO, at the same time period, suggests a selective vasoconstrictive impact of thermal trauma on the hepatic arterial circulation.

The second insult (LPS) yielded a dramatic and prolonged increase in HAVR (16-fold vs. baseline), which was associated with a significant reduction of Qh during the first 4 hours after endotoxin (12%-67% of baseline). After recovery to baseline, a reperfusion episode followed with an elevation of Qh to 152% of baseline values at 8 hours after endotoxin.

The impact of endotoxin on Qh, as observed in this study, is more pronounced compared with the results of a previous study [11]. The effect of endotoxemia on the hepatic circulation was evaluated in chronically instrumented and sedated sheep receiving a continuous intravenous infusion of *E. coli* endotoxin [11]. The response of the hepatic artery was biphasic and consisted of a transient vasoconstriction followed by a transitory increase of Qh, reaching a maximum of 921% of baseline values after approximately 2 hours. The noticed dissimilarity might be due to the fact that different species and administration schemes were used. However, it is more likely that the priming impact of the early burn injury was responsible for the amplification of Qh response to endotoxin.

Under physiological conditions, the regulation of Qh tends to buffer the impact of Qp changes on total hepatic blood flow in order to maintain the latter constant. The function of Qp as the major intrinsic regulator of hepatic arterial tone is known as the hepatic arterial buffer response [12]. This buffer function seems to depend on portal blood flow washing away local concentrations of adenosine from the area of arterial resistance [13]. Thus, a reduction in portal blood flow causes an increase in local adenosine levels, resulting in arterial dilation [13]. Since Qp is determined by the vascular resistance of the intestine and, to a lesser extent, the spleen and pancreas, a decrease in the mesenteric blood flow would result in a compensatory increase in hepatic blood flow to maintain liver function.

In contrast with this scenario, in our model of postburn endotoxemia, the response of Qh was unrelated to changes in portal circulation. An early postburn transient hepatic vasoconstriction was found to occur before changes in portal circulation (1 hour vs. 2 hours after burn), demonstrating the relative independence of hepatic arterial response in relation with other splanchnic blood flow. This ischemic insult may explain the occurrence of transient hepatic function disorders, commonly seen early after burn, and may also adversely influence the phagocytic capacities of hepatic macrophages. It is also possible that this hepatic ischemic insult may prime hepatic macrophages, leading to release of certain vasomediators, such as thromboxanes, which have been implicated in the pathophysiologic mechanism of postburn mesenteric vasoconstriction [14]. Despite the marked reduction in Qp during the first 8 hours after endotoxin, Qh showed a significant decrease for a period of 4 hours, indicating a loss of the hepatic arterial buffer response. The magnitude of the hepatic response after the second insult may demonstrate the priming effect of the first insult. In an intact porcine model of endotoxemia, Ayuse et al. have observed a similar alteration in the hepatic arterial buffer response [15]. It has been suggested that certain mediators, gut-derived or generated by presinusoidal Kupffer cells, are implicated in the pathophysiologic process of the impaired hepatic arterial buffer response [11]. Another possible mediator could be nitric oxide (NO), because it has been reported to be involved in the pathophysiology of liver injury during ischemia/reperfusion and endotoxemia [16-18]. It has been suggested that endogenous NO formation is sufficient to limit ischemic liver injury during reperfusion and that inhibition

of NO synthesis will result in additional ischemic damage [16]. NO has been reported to modulate hepatic arterial but not portal venous resistances under baseline conditions, induce hepatic arterial vasodilation, and attenuate the increase in portal resistance during endotoxic shock [17]. Another experimental study has demonstrated that inhibition of eNOS (L-NAME) has a detrimental effect on liver injury during ischemia/reperfusion and endotoxemia, mainly because it can cause additional ischemia by reducing the microvascular blood flow [18].

The marked portal hypertension, seen in the postburn endotoxemic period, may account for the occurrence of bacterial translocation and contribute to 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 [15,22]. Portal hypertension initiated by endotoxin has also been shown to induce hepatic microcirculatory disturbance, which may cause liver injury [23].

It is well known that oxygen delivery and utilization is deranged in the setting of sepsis. Patients with septic shock require higher levels of DO_2 to maintain aerobic metabolism. When DO_2 is inadequate, peripheral tissues switch to anaerobic metabolism and VO_2 decreases [24]. The most likely cause is an inability of the microvasculature to provide sufficient oxygen to actively metabolic tissue, probably as a result of diminished autoregulatory control and capillary damage [25].

In our study, DO_2 and VO_2 showed a similar pattern of increasing, as seen in CO following thermal injury. On the contrary, hDO_2 was significantly reduced during this early postburn phase. At the same time, hVO_2 did not show any significant changes, as evidence of an increased oxygen extraction capacity of hepatic cells. It is well known that the liver can extract nearly 100% of the available oxygen [26]. This process seems to be the primary compensatory adjustment after a reduced hDO_2.

The impact of this second insult on the hepatic oxygenation was dramatic. A pathologic flow-dependent hVO_2 response was observed, with a pronounced and prolonged hypoxic period. The first 4 hours after LPS administration to burned animals

were marked with a significant decrease in hDO_2. Hepatic oxygen consumption showed a pathologic hDO_2 dependency, leading to an oxygen debt that limits metabolism. This early decreased hVO_2 indicates the inability of the liver to compensate inadequate 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 DO_2 in accordance with tissue oxygen needs [27]. 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 [28]. Early hypoxia in the splanchnic region is suggested as a plausible mechanism behind the development of secondary organ failure, especially in sepsis [28]. Recently, NO has been shown to be involved in hepatic oxygen transport and consumption during endotoxemia [19,29]. The role of NO in hepatic oxygen transport is unclear. In a porcine model of endotoxemia, NO was found to ameliorate deterioration of hepatic oxygen transport and liver function induced by endotoxin [29]. In another study, selective inhibition of iNOS activity was found to restore Qh and increase hVO_2 in pigs with endotoxemia [19].

In conclusion, thermal injury appears to induce a selective vasoconstrictive effect on the hepatic arterial circulation, yielding hepatic ischemia and a reduction of hepatic oxygen delivery. The magnitude of the hepatic response to a second insult (endotoxemia) is magnified and manifested as a pronounced hepatic ischemia and reperfusion episode, associated with an inadequate hepatic oxygen delivery and a pathologic supply-dependent hepatic oxygen consumption. A loss of the hepatic arterial buffer response, as indicated by a profound decline in both portal venous and hepatic arterial blood flow, seems to be one of the mechanisms responsible for this phenomenon. In addition, postburn endotoxemia seems to induce severe portal hypertension, which may contribute to gut barrier dysfunction following thermal injuries.

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CHAPTER 8

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

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Abstract

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.

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 (LPS) was intravenously administered at 100 μg/kg 18 hours postburn. DuP753 was administered intravenously at 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 14 consecutive time points, starting 1 hour after the burn.

Results: Burn caused a 4.6-fold increase in hepatic arterial vascular resistance (HAVR) and a 49% decrease in hepatic arterial blood flow (HABF). Postburn administration of angiotensin II receptor blocker DuP753 yielded a significant improvement in the hepatic arterial hemodynamics (only 12% increase in HAVR and 8% decrease in HABF, $P<0.05$ vs. nontreated group, analysis of variance [ANOVA]). Post-LPS, HABF was significantly reduced (12% of baseline, $P<0.05$, ANOVA), in contrast with DuP753-treated animals (64% of baseline, $P<0.05$ vs. nontreated group, ANOVA). Postburn blocking of angiotensin II receptors yielded a significant improvement in post-LPS portal venous blood flow (PVBF, 85% of baseline vs. 48% of baseline in nontreated animals, $P<0.05$, ANOVA). Postburn endotoxemia resulted in a significant decrease of hepatic oxygen delivery (hDO_2, 22% of baseline) and hepatic oxygen consumption (hVO_2, 30% of baseline), while no marked changes were observed in the DuP753-treated group ($P<0.05$ 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 impact of thermal injuries and sepsis on hepatic perfusion and oxygenation.

Introduction

Although sepsis and multiple organ dysfunction syndrome (MODS) 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 post-injury 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]

The results of our previous studies in a porcine model[4] 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 and reperfusion injury, associated with an inadequate hepatic oxygen delivery (hDO_2) and a pathologic supply-dependent hepatic oxygen consumption (hVO_2).[4,5] In this study an attempt was made to investigate the role of angiotensin II as a possible mediator involved in this process.

Materials and Methods

The following experimental protocols were approved by the Animal Care and Use Committee of the University of Texas Medical Branch, Galveston (ACUC #90-09-103).

Eighteen female mini-pigs, weighing between 20 and 25 kg, were prepared surgically 5 days before the experiment. After an overnight fast, the pigs were anesthetized with intramuscular ketamine (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 and the left hepatic vein. A Witzel jejunostomy was also performed using a 12F Foley catheter. The abdomen was closed in layers.

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, PR) was positioned in the pulmonary artery via 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/hr and nothing orally. Baseline data were collected after complete recovery from anesthesia.

The Pigs were randomized into the following 3 groups:

1) The burn/lipopolysaccharide (LPS) group (n=6) had a 40% total body surface area (TBSA) third-degree flame burn under general anesthesia, as described before.[10] The pigs were resuscitated according to the Parkland formula and received lactated Ringer's solution, 4 mL/kg/%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 *E. coli* LPS (0111:B4; Difco, Detroit, MI) was intravenously administered. During the second day of the experiment, burned

animals received lactated Ringer's solution at 3,5 mL/m^2 burned area and 2 mL/kg/hr for daily maintenance.

2) The sham group (n=6) had a sham burn under anesthesia. Eighteen hours later the animals received the diluent (0.9% NaCl) used for the endotoxin. Lactated Ringer's solution was administered at 2 mL/kg/hr for daily maintenance.

3) The treatment group (n=6) underwent the same procedure as the burn/LPS group, except for the administration of angiotensin II inhibitor DuP753 (DuPont Merck, Wilmington, DE) intravenously at 1 μg/kg, immediately after burn.

Mean arterial (MAP), central venous (CVP) and portal venous (PVP) pressures were measured using transducers (P231D, Statham Gould, Oxnard, CA) that were connected to an Electronic Medicine Honeywell Recorder (Honeywell Inc., Pleasantville, NY) for electronic calculation of mean pressures. Cardiac output (CO) was determined by the thermal dilution technique using a Swan-Ganz catheter and a cardiac output computer (Model 9520, American Edwards Laboratories, Irvine, CA).

Hepatic arterial blood flow (HABF) and hepatic portal venous blood flow (PVBF) were measured with transit time ultrasonic flow probes, connected to an ultrasonic meter (T101, Transonic Systems Inc., Ithaca, NY).

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

Systemic vascular resistance index (SVRI), hepatic arterial vascular resistance (HAVR), and hepatic portal vascular resistance (HPVR) were calculated with the following formulas:

SVRI (Dyne·second·cm^{-5}·m^2)=[(MAP - CVP) x 80]/CI

HAVR (Dyne·second·cm^{-5})=[(MAP - CVP) x 80]/Qh

HPVR (Dyne·second·cm^{-5})=[(PVP - CVP) x 80]/Qp

Systemic O_2 delivery (DO_2), systemic O_2 consumption (VO_2), hepatic O_2 delivery (hDO_2) and hepatic O_2 consumption (hVO_2) were calculated with the following formulas:

DO_2=CI x CaO_2 x 10 (mL/min/m^2)

VO_2=CI x (CaO_2 - CvO_2) x 10 (mL/min/m^2)

hDO_2 =HABF x CaO_2 / 100 (mL/min)

hVO_2 =HABF x (CaO_2 - ChO_2) / 100 (mL/min)

Where CI= cardiac index (L/min/m^2)=CO/BSA; CaO_2 (arterial oxygen content, mL/dL)= (Hb x 1.34) SaO_2 [arterial oxygen saturation] + (PaO_2 x 0.0031); CvO_2 (mixed venous oxygen content, mL/dL)= (Hb x 1.34) SvO_2 [mixed venous oxygen saturation] + (PvO_2 x 0.0031); and ChO_2 (hepatic oxygen content, mL/dL)= (Hb x 1.34) ShO_2 [hepatic blood oxygen saturation] + (PhO_2 x 0.0031).

At the end of the 42 hours, the animals were anesthetized with 10 mg/kg intravenous ketamine and killed with 5 mL intravenous saturated KCl.

The data are presented as mean ± SEM. Within-group analysis was performed by the analysis of variance (ANOVA) for repeated measurements with the Dunnet post hoc test. Between-groups analysis was performed by ANOVA for factorial analysis with the bonferroni post hoc test. $P < 0.05$ was considered statistically significant.

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 (Fig. 1 and Fig. 2) and hepatic (Fig. 3 and Fig. 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 (Fig. 1). This increase was associated with a concomitant fall in the systemic vascular resistance, whereas the systemic vascular resistance index (SVRI) decreased to 78% of baseline level (Fig. 1). No significant differences were noticed in MAP and CVP between the 3 groups (Fig. 2).

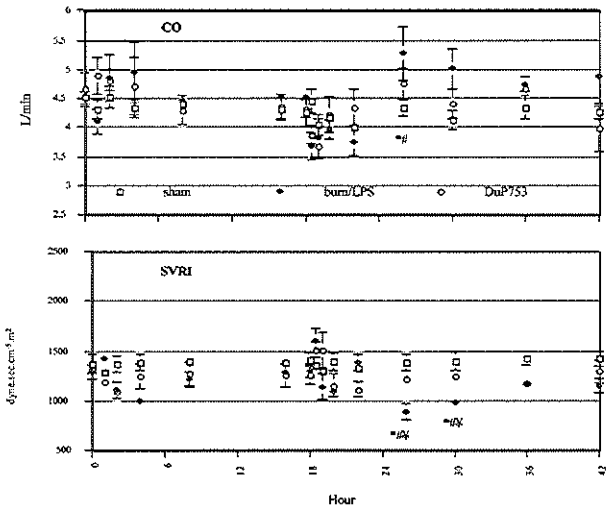

Figure 1. Systemic hemodynamics 1: cardiac output (CO) and systemic vascular resistance index (SVRI) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. Coli* LPS, 18 hours after burn). Angiotensin II inhibitor DuP753 was administered intravenously immediately after burn. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

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 a hyperdynamic period began to be manifest 8 hours after

endotoxin administration (Fig. 1). At this time point, SVRI dropped to 69% of baseline. MAP showed a 14% decrease immediately after LPS infusion in both burn/LPS groups (Fig. 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 (Fig. 1 and Fig. 2)

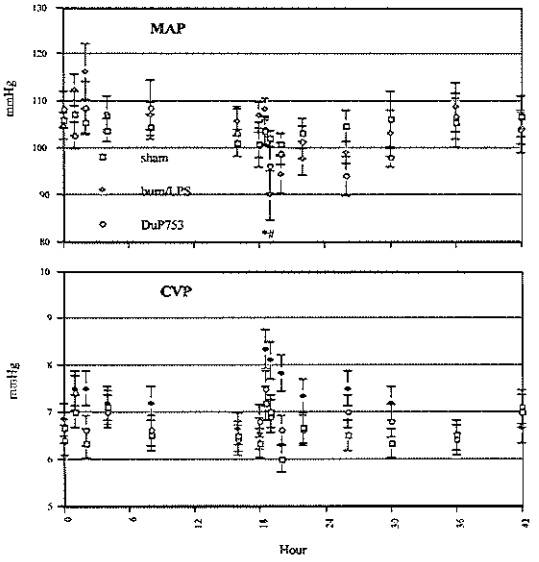

Figure 2. Systemic hemodynamics 2: mean arterial pressure (MAP) and central venous pressure (CVP). $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

Hepatic Hemodynamics

Hepatic Arterial Circulation

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

Compared with burned animals not receiving the angiotensin II antagonist, DuP753-treated animals showed a slight increase of HAVR (12% of baseline value) following thermal insult (Fig. 4). Animals in the DuP753 treatment group maintained, in contrast to

nontreated burned animals, a stable HABF near baseline with an average fall of 8% of baseline (Fig. 3).

Hepatic arterial hemodynamic measurements recovered completely in all animals to baseline values at 18 hours after the burn.

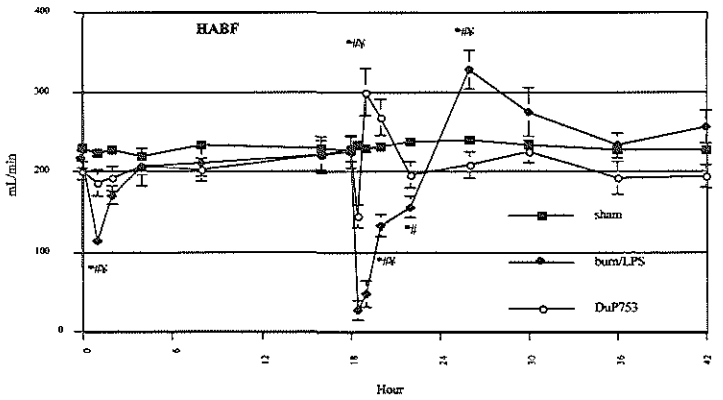

Figure 3 Burn caused a 49% decrease in hepatic arterial blood flow (HABF). Post-LPS HABF was significantly reduced (12% of baseline). Postburn administration of angiotensin II receptor blocker DuP753 yielded a significant improvement in the hepatic arterial hemodynamics ($P<0.05$ vs. nontreated group, ANOVA). $P<0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

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 16-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 (Fig. 3 and Fig. 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 in 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 min.), where after both HAVR and HABF measurements were within baseline range (Fig. 3 and Fig. 4).

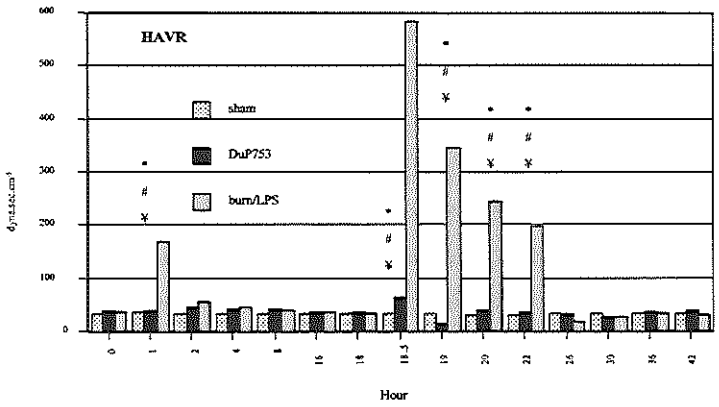

Figure 4. Burn and LPS yielded a significant increase (4.6- and 16-fold, respectively) in hepatic arterial vascular resistance (HAVR). DuP753 treatment ameliorated these effects. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

Hepatic Portal Circulation

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

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

The second insult yielded significant alterations in the portal circulation, lasting for a prolonged period. HPVR showed a 2- to 4-fold increase during the first 8 hours following LPS administration (Fig. 6). During this early septic phase, a significant portal hypertension was noticed, whereas measured PVP was elevated to approximately 225% of baseline (Fig. 7). PVBF showed a biphasic response after LPS administration. During the first 8 hours after LPS administration, PVBF decreased to approximately 51% of baseline (Fig. 5). 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 till the end of the study period (Fig. 5). During this late septic period, HPVR was decreased to 63% of baseline and PVP was slightly increased to 121% of baseline (Fig. 6 and Fig. 7). DuP753 treatment significantly attenuated the impact of the second insult (LPS) on portal hemodynamics in burned animals. In contrast with nontreated 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 (Fig. 6). DuP753 treatment ameliorated the LPS-induced portal ischemia and 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 (Fig. 5). Portal hypertension did not occur after LPS administration to burned animals treated with DuP753. PVP remained within baseline range and a slight increase of 18% was noticed during the first 4 hours after LPS administration (Fig. 7).

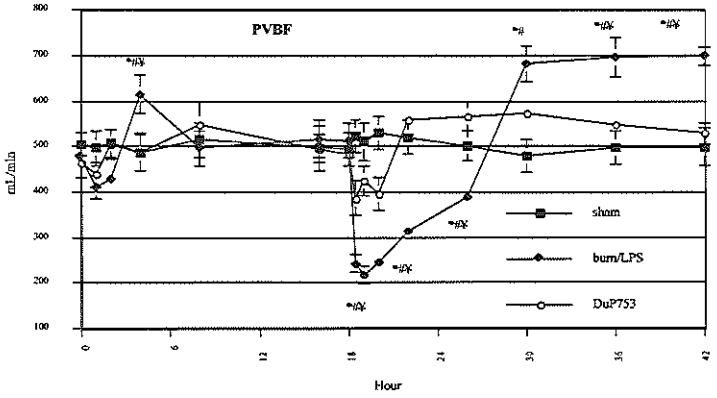

Figure 5. Postburn blocking of angiotensin II receptors yielded a significant improvement in post-LPS portal venous blood flow (PVBF, 85% of baseline vs. 48% of baseline in nontreated animals, $P<0.05$, ANOVA). $P<0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

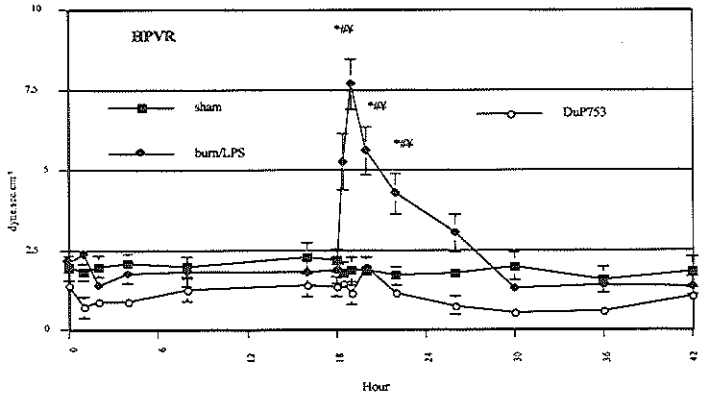

Figure 6. Hepatic portal vascular resistance (HPVR) in the 3 experimental groups. $P<0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

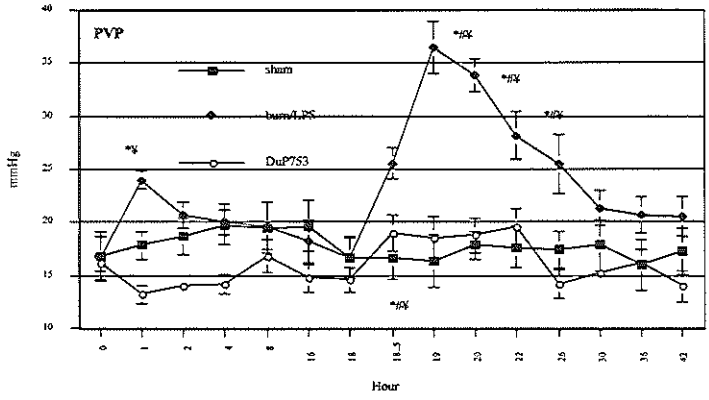

Figure 7. Portal venous pressure (PVP) was significantly elevated after burn and endotoxin, but not in the treatment group. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

Systemic Oxygen Delivery and Consumption

After an initial reduction, DO_2 and VO_2 showed a marked increase during the first 4 hours after the burn (Fig. 8).

Administration of LPS yielded a significant drop in DO_2 during the first hour. VO_2 was unchanged at this time point. Animals treated with DuP753 remained at baseline after LPS administration (Fig. 8). During the post-LPS hyperdynamic phase, both DO_2 and VO_2 were increased.

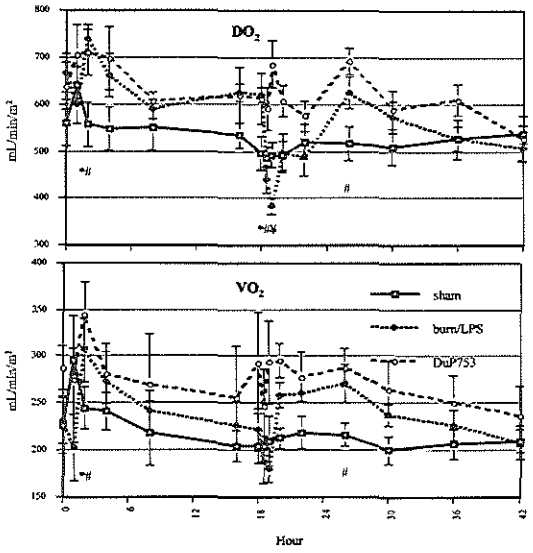

Figure 8. Effects of burn, endotoxin and postburn administration of angiotensin II inhibitor DuP753 on systemic oxygen delivery (DO_2) and oxygen consumption (VO_2). $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

Hepatic Oxygen Delivery and Consumption

During the first 2 hours after the burn, hDO_2 showed a significant drop to 53% of baseline, while hVO_2 decreased to 15% of baseline levels (Fig. 9). In contrast, DuP753-treated animals did not show any marked alterations in hDO_2 and hVO_2 after thermal injury (Fig. 9).

The second insult (LPS) yielded a dramatic reduction in hDO_2 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 (Fig. 9). Postburn treatment with DuP753 prevented this impact of LPS, as hDO_2 showed a transient 16% increase at 1 hour after LPS administration and then remained at baseline. hVO_2 showed a similar pattern, whereas a significant decrease of 30% to 63% of baseline was calculated during the first 4 hours after LPS administration, followed by a 50% increase at 8 hours after LPS administration (Fig. 9). Animals receiving DuP753 did not show any significant changes in their hVO_2 after LPS administration (Fig. 9).

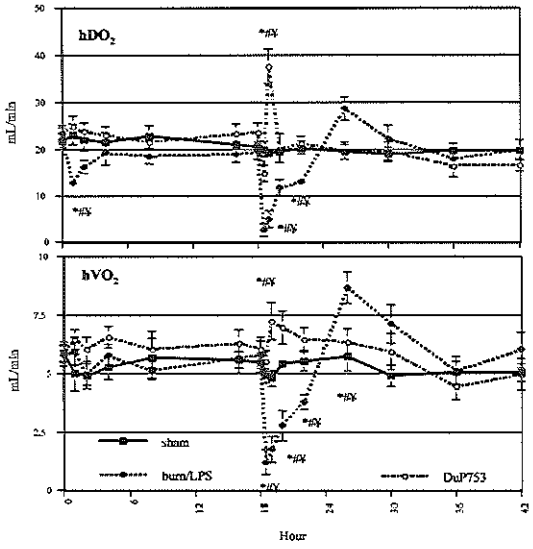

Figure 9. Hepatic oxygen delivery (hDO_2) and hepatic oxygen consumption (hVO_2) showed a significant drop after burn and endotoxin. DuP753 treatment significantly attenuated the impact of both insults. P <0.05, * vs. baseline, # vs. sham, ¥ vs. treatment group (DuP753).

Comment

The renin-angiotensin axis seems to play an important role in the pathophysiology of thermal injury. Hilton et al[7] 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.[8]

Our current data confirm our previous study reporting the alteration of hepatic hemodynamics secondary to thermal injury.[4] Despite indicators of adequate resuscitation (i.e. minor changes in CO, MAP, and CVP), a significant reduction in HABF was observed in this study during the early phase after thermal injury. Thus, alteration in systemic hemodynamics could not be solely accounted for the postburn hepatic arterial vasoconstrictive phase that was noticed. The finding that no noticeable changes were seen in either HABF or HAVR after thermal injury in the group treated with angiotensin II receptor blocker DuP753, implicates angiotensin II in the process of postburn hepatic ischemia. The significant improvement of postburn HABF following DuP753 treatment, despite no marked DuP753 systemic effects, suggests a selective action on splanchnic vasculature.

The response of the hepatic arterial blood flow to the initial thermal trauma was unrelated to changes in portal circulation. The early postburn transient hepatic vasoconstriction occurred simultaneously with a decrease in the portal circulation, indicating an early loss of the hepatic arterial buffer response. Under physiological conditions, the regulation of HABF tends to buffer the impact of PVBF changes on total hepatic blood flow in order to maintain the latter constant. The function of PVBF as the major intrinsic regulator of hepatic arterial tone is known as the hepatic arterial buffer response.[9] This buffer function seems to depend on portal blood flow washing away local concentrations of adenosine from the area of the arterial resistance site.[10] The selective improvement in hepatic arterial circulation 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 impact of the second septic insult on both hepatic arterial and portal venous circulations. The noticed decrease in both arterial and portal hepatic blood flow after LPS administration cannot be the result of alterations in the systemic circulation, as post-endotoxin 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 post-injury 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 flow[6] 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 II receptor blocker DuP753 ameliorated this process, indicates that angiotensin II is one of the mediators responsible for the adverse impact 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]

LPS-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 to be an endogenous nitrovasodilator,[17] by inhibiting induction of the 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 II receptor blocking treatment was the prevention of the sepsis-induced portal hypertension. Postburn endotoxemia-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 current study, hDO_2 was significantly reduced during this early postburn phase. DuP753 treatment significantly improved hDO_2 after thermal injury.

The second insult yielded a significant fall in hDO_2rate. A pathologic flow-dependent hDO_2 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.[25] hVO_2 showed a pathologic hDO_2 dependency, leading to oxygen debt that limits metabolism. This early decreased hVO_2 indicates the inability of the liver to compensate inadequate 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 DO_2 in accordance 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.[16,27] 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 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 DO_2 and VO_2.

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.

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CHAPTER 9

Opposite Effects of Prostacylin on Hepatic Blood Flow and Oxygen Consumption after Burn and Sepsis

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Abstract

Objective: to examine the hypothesis that postburn treatment with the vasodilator prostacyclin would be beneficial for hepatic perfusion and oxygenation in a burn/endotoxemia chronic porcine model.

Summary Background Data: Burn and sepsis are associated with hepatic ischemia and reperfusion injury. Prostacyclin, the production of which has been reported to decrease after burn, seems to play a pivotal role in maintaining splanchnic perfusion.

Methods: Female pigs (n=18, 20-25 kg) underwent laparotomy during which ultrasonic flow probes were placed 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. 100 μg/kg *Escherichia coli* lipopolysaccharide (LPS) was intravenously administered 18 hours postburn. Burned animals were randomized to receive a constant infusion of iloprost (20 ng/kg/min) or an equivalent amount of carrier solution (normal saline). All animals were studied for 42 hours.

Results: Burn caused a 2.5-fold increase in hepatic arterial vascular resistance (HAVR) and a 39% decrease in hepatic arterial blood flow (HABF). Postburn administration of iloprost did not improve the hepatic arterial hemodynamics (1.8-fold increase in HAVR and 38% decrease in HABF). Post-LPS, HABF was significantly reduced to 22% of baseline and HAVR was 15-fold increased ($P<0.05$ vs. baseline, ANOVA). In contrast, iloprost-treated animals did not show hepatic arterial vasoconstriction, as both HABF and HAVR remained baseline values during the endotoxic phase, ($P<0.05$ vs. nontreated group, ANOVA). Postburn iloprost treatment yielded a significant improvement in post-LPS portal venous blood flow (PVBF, 79% of baseline vs. 45% of baseline in nontreated animals, $P<0.05$, ANOVA). Portal venous pressure (PVP) showed 16% and 56% increases after burn and endotoxin, respectively. Portal hypertension did not occur in iloprost-treated animals, as PVP remained within baseline range ($P<0.05$ vs. nontreated group, ANOVA). Burn and endotoxemia resulted in a significant decrease of hepatic oxygen delivery (hDO_2, 63% and 12% of baseline, respectively) and hepatic oxygen consumption (hVO_2, 61% and 21% of baseline, respectively). Only during the postburn endotoxic phase, iloprost improved hDO_2 and hVO_2 (140% and 79%, respectively. $P<0.05$ vs. nontreated group, ANOVA).

Conclusions: Postburn prostacyclin treatment appears to have no beneficial effects on hepatic perfusion early postburn. However, during the late postburn endotoxic phase prostacyclin seems to significantly improve hepatic total blood flow and oxygenation. In addition, prostacyclin treatment attenuated burn- and endotoxin- induced portal hypertension.

Introduction

The prognosis of extensively burned patients is dependent upon the presence of sepsis and/or multiple organ failure. The splanchnic organs are considered to be one of the key components in the pathogenesis of such complications [1, 2]. Disturbances in organ blood flow are suggested to play a pivotal role in the development of progressive organ dysfunction [3]. While restoration of optimal perfusion is essential in reducing both initial ischemia-induced injury and subsequent progressive metabolic and structural derangement, further cell deterioration might occur following reperfusion [4].

The data of our previous studies in a porcine model showed that thermal injury has a selective vasoconstrictive effect on the hepatic arterial circulation. Moreover, a subsequent insult (endotoxemia) was found to induce a significant hepatic ischemia and reperfusion injury, associated with an inadequate hepatic oxygen delivery and a pathologic supply-dependent hepatic oxygen consumption [5, 6].

Endogenous prostacyclin is known to be a local mediator of microcirculatory tissue perfusion and metabolism and is of major importance in maintaining splanchnic perfusion after different endogenous vasoconstrictive stimuli, both in animals and in humans [7, 8]. In both septic animals [9] and patients after mesenteric traction during major abdominal surgery [10], the endogenous release of prostacyclin proved to play a crucial role in maintaining splanchnic perfusion.

In this study we examined the hypothesis that the stable prostacyclin analogue iloprost may beneficially influence the hepatic hemodynamics and thereby improve tissue perfusion and oxygenation in a burn/endotoxin porcine model.

Materials and Methods

The following experimental protocols were approved by the Animal Care and Use Committee of the University of Texas Medical Branch (ACUC # 90-09-103).

Eighteen female mini-pigs, weighing between 20 and 25 kg, were prepared surgically 5 days before the experiment. After an overnight fast, the pigs were anesthetized with intramuscular ketamine (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. 6.5 F catheters were positioned in the superior mesenteric vein and the left hepatic vein. A Witzel jejunostomy was also performed using a 12 F Foley catheter. The abdomen was closed in layers.

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, PR) was positioned in the pulmonary artery via the right jugular vein. A 12 F 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/hr and nothing per os. Baseline data were collected after complete recovery from anesthesia.

The pigs were randomized into 3 groups:

1) The burn/lipopolysaccharide (LPS) group (n=6) had a 40% total body surface area (TBSA) third-degree flame burn under general anesthesia, as described before [11]. The pigs were resuscitated according to the Parkland formula and received lactated Ringer's solution, 4 mL/kg/%TBSA burn, starting immediately after the burn, half of which was given in the first 8 hours postburn and the remainder in the following 16 hours. Eighteen hours after burn, 100 μg/kg *E. coli* LPS (0111:B4; Difco, Detroit, MI) was intravenously administered. During the second day of the experiment, the burned animals received lactated Ringer's solution at 3,5 mL/m^2 burned area and 2 mL/kg/hr for daily maintenance.

2) The sham group (n=6) had a sham burn under anesthesia. Eighteen hours later the animals received the diluent (0.9% NaCl) used for the endotoxin. Lactated Ringer's solution was administered at 2 mL/kg/hr for daily maintenance.

3) The treatment group (n=6) underwent the same procedure as the burn/LPS group, except for the administration of prostacylin analogue iloprost (Ilomedin, Schering, AG, Germany) intravenously at 20 ng/kg/min, immediately after burn.

Mean arterial (MAP), central venous (CVP) and portal venous (PVP) pressures were measured using transducers (P231D, Statham Gould, Oxnard, CA) that were connected to an Electronic Medicine Honeywell Recorder (Honeywell Inc., Plaeasantville, NY) for electronic calculation of mean pressures. Cardiac output (CO) was determined by the thermal dilution technique, using a Swan-Ganz catheter and a cardiac output computer (Model 9520, American Edwards Laboratories, Irvine, CA).

Hepatic arterial blood flow (HABF) and hepatic portal venous blood flow (PVBF) were measured with transit time ultrasonic flow probes, connected to a T101 ultrasonic meter (Transonic Systems Inc., Ithaca, NY).

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

Systemic vascular resistance index (SVRI), hepatic arterial vascular resistance (HAVR), and hepatic portal vascular resistance (HPVR) were calculated with the following formulas:

SVRI (dyne·sec·cm^{-5}·m^2)=[(MAP - CVP) x 80] / CI

HAVR (dyne·sec·cm^{-5})=[(MAP - CVP) x 80] / Qh

HPVR (dyne·sec·cm^{-5})=[(PVP - CVP) x 80] / Qp

Systemic oxygen delivery (DO_2), systemic oxygen consumption (VO_2), hepatic oxygen delivery (hDO_2) and hepatic oxygen consumption (hVO_2) were calculated with the following formulas:

DO_2=CI x CaO_2 x 10 (mL/min/m^2)

VO_2=CI x (CaO_2 - CvO_2) x 10 (mL/min/m^2)

hDO_2 =HABF x CaO_2 / 100 (mL/min)

hVO_2 =HABF x (CaO_2 - ChO_2) / 100 (mL/min)

Where CI= cardiac index (L/min/m^2)=CO/BSA, CaO_2 (arterial oxygen content, mL/dL)= (Hb x 1.34) SaO_2 + (PaO_2 x 0.0031), CvO_2 (mixed venous oxygen content, mL/dL)= (Hb x 1.34) SvO_2 + (PvO_2 x 0.0031), and ChO_2 (hepatic oxygen content, mL/dL)= (Hb x 1.34) ShO_2 + (PhO_2 x 0.0031).

At the end of the 42 hours, the animals were anesthetized with 10 mg/kg intravenous ketamine and killed with 5 mL intravenous saturated KCl.

The data are presented as mean ± SEM. Within-group analysis was performed by the analysis of variance (ANOVA) for repeated measurements with the Dunnet post hoc test. Between-groups analysis was performed by ANOVA for factorial analysis with the bonferroni post hoc test. *P* values of <0.05 were considered statistically significant.

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 (Figs. 1 and 2) and hepatic (Figs. 3 and 5) hemodynamics within baseline range.

Thermal injury reduced CO to 72% of baseline level during the first hour (Fig. 1). By the second hour postburn, a hyperdynamic phase began with an increase in CO to 139% of baseline. This increase was associated with a concomitant fall in the systemic vascular resistance, whereas SVRI decreased to 81% of baseline (Fig. 1). There were no significant differences in MAP and CVP between the 3 groups (Fig. 2).

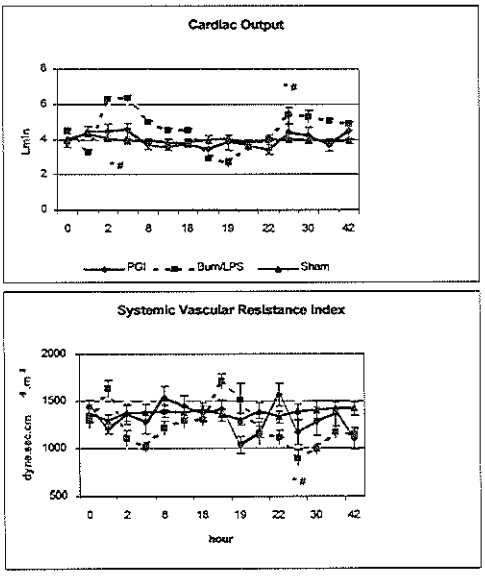

Fig 1. Systemic hemodynamics 1: cardiac output (CO) and systemic vascular resistance index (SVRI) after 40% TBSA third-degree burn (0 hour) and endotoxin (*E. coli* LPS, 18 hours postburn). Prostacyclin (PGI_2) analogue iloprost was administered intravenously immediately after burn. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

Following administration of LPS, a typical biphasic response was observed. After a marked drop of CO to 64% of baseline level, a hyperdynamic period began to be manifest 8 hours post-endotoxin (Fig. 1). At this time point, SVRI dropped to 76% of baseline. MAP showed a 27% decrease immediately after LPS infusion in both burn/LPS

groups (Fig. 2). During the further post-LPS course, no significant differences were noticed between groups in MAP and CVP. Iloprost treatment attenuated to a certain extent the alteration in systemic circulation occurring after burn and LPS administration (Figs. 1 and 2).

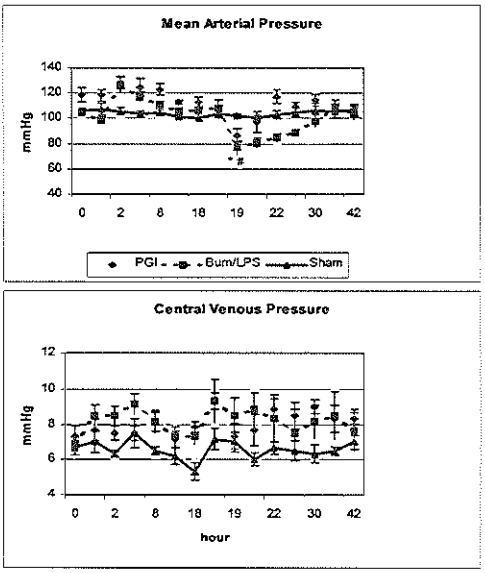

Fig 2. Systemic hemodynamics 2: mean arterial pressure (MAP) and central venous pressure (CVP). $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

Hepatic Hemodynamics:

Hepatic arterial circulation:

During the first 4 hours postburn, HABF was significantly reduced to approximately 61% of baseline level (Fig. 3). This fall in HABF was associated with a 2.5-fold increase in HAVR as early as 1 hour postburn (Fig. 4).

Iloprost-treated animals showed a 1.8-fold increase in HAVR following thermal insult (Fig. 4). HABF showed a significant reduction of approximately 38% of baseline level in iloprost-treated animals. The alteration in hepatic arterial hemodynamic parameters lasted for the whole postburn phase in the iloprost-treated group. Hepatic arterial hemodynamic measurements recovered completely in all nontreated animals to baseline values at 18 hours postburn.

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 15-fold increase of HAVR during the first hour post-LPS.

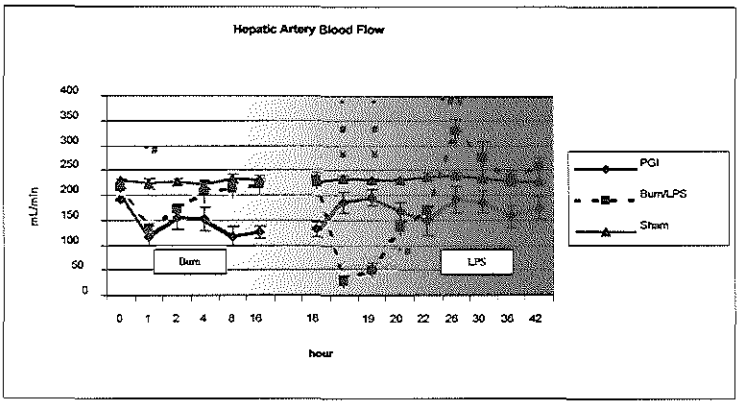

Fig 3. Hepatic arterial blood flow (HABF) was significantly decreased in both burn/LPS and iloprost groups. Postburn administration of iloprost yielded a significant improvement in HABF following endotoxin (P<0.05 vs. nontreated group, ANOVA). P <0.05, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

Correspondingly, HABF decreased significantly to 22% of baseline during the same time of maximum increase of HAVR (Figs. 3 and 4). After an initial recovery of HABF to baseline values 6 hours post-LPS, a marked elevation (148% of baseline) was noticed during the following 6 hours.

Burned animals treated with iloprost maintained baseline level during the next 24 hours after the second insult (LPS). Iloprost treatment attenuated significantly the hepatic arterial vasoconstriction caused by the administration of LPS to burned animals, as HAVR did not increase but remained within baseline range to the end of the study (Figs. 3 and 4).

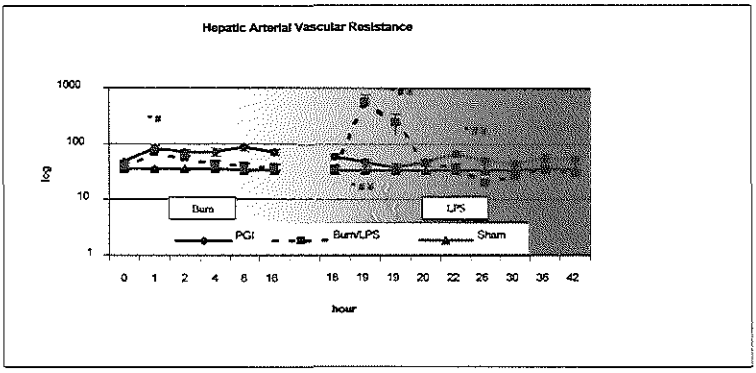

Fig 4. Burn and LPS yielded a significant increase (2.5- and 15-fold, respectively) in hepatic arterial vascular resistance (HAVR). Iloprost treatment ameliorated these effects only during the late postburn endotoxic phase. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

Hepatic portal circulation:

During the first 4 hours postburn, PVBF showed an increase, reaching 130% of baseline at 2 hours postburn (Fig. 5). The increased PVBF was associated with a moderate decrease in HPVR (68% of baseline) during the same time period (Fig. 6). PVP showed a 16% increase 1 hour postburn and declined thereafter to baseline level during the next 4 hours postburn (Fig. 7). In contrast, animals treated with iloprost showed a moderate decrease in their postburn PVBF of approximately 20% of baseline values (Fig. 5). HPVR in the treatment group increased to 161% of baseline values during the first 4 hours postburn (Fig. 6). PVP did not show any changes in iloprost- treated animals during the early postburn phase (Fig 7).
Portal hemodynamic measurements showed a recovery to baseline values at 18 hours postburn in all animals (Figs. 5, 6 and 7).

The second insult yielded significant alterations in the portal circulation, lasting for a prolonged time period. HPVR showed a 3.6-fold increase during the first 8 hours following LPS administration (Fig. 6). During this early septic phase, a significant portal

hypertension was noticed, whereas measured PVP was elevated to approximately 156% of baseline values (Fig. 7).

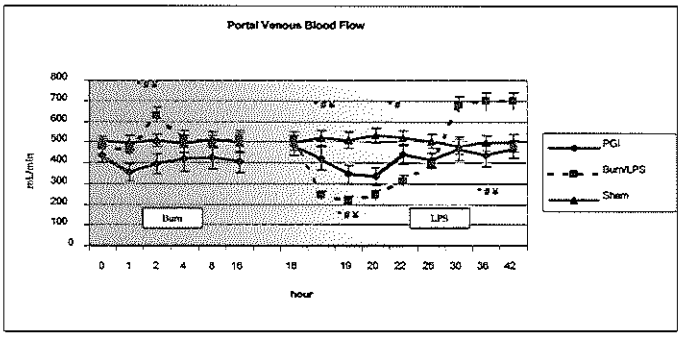

Fig 5. Burn and endotoxin resulted in significant alterations in portal venous blood flow (PVBF). Iloprost-treated animals showed steady PVBF values within baseline. P <0.05, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

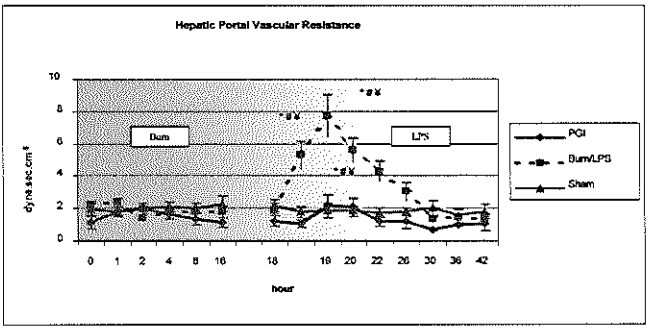

Fig 6. Postburn endotoxemia yielded a significant increase (3.6-fold) in hepatic portal vascular resistance (HPVR). Iloprost treatment ameliorated this effect. P <0.05, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

PVBF showed a biphasic response after LPS administration. During the first 8 hours post-LPS, PVBF decreased to approximately 45% of baseline (Fig. 5).
After a transit recovery to baseline, a hyperdynamic phase with an elevation of PVBF to 141% of baseline began at 30 hours (12 hours post-LPS) and remained till the end of the study period (Fig. 5). During this late septic period, HPVR was decreased to 61% of baseline and PVP was stable at baseline level (Figs. 6 and 7). Iloprost treatment significantly attenuated the impact of the second insult (LPS) on portal hemodynamics in burned animals. In contrast to nontreated animals, HPVR in the treated group showed for a short period of 2 hours an increase of 1.8-fold, followed by a steady period of 22 hours at baseline level (Fig. 6). Iloprost treatment ameliorated the LPS-induced portal ischemia and reperfusion insult in burned animals, as PVBF showed after a moderate decrease of 21% at 2 hours post-LPS, a steady increase within baseline range during the rest of the study period (Fig. 5). Portal hypertension did not occur after LPS administration to burned animals treated with iloprost. PVP remained within baseline range (Fig. 7).

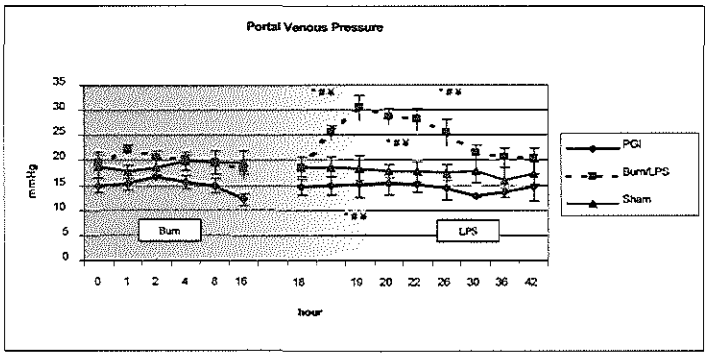

Fig 7. Portal venous pressure (PVP) was significantly elevated after burn and endotoxin, but not in the treatment group. $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

Systemic Oxygen Delivery and Consumption:

After an initial reduction during the first 2 hours postburn, DO_2 and VO_2 showed a marked increase during the following 6 hours postburn (Fig. 8). Both DO_2 and VO_2 were elevated in the iloprost treatment group during the first 8 hours postburn.

The second insult (LPS) yielded a significant drop in DO_2 during the first 2 hours. VO_2 remained unchanged at this time point. During the post-LPS hyperdynamic phase, both DO_2 and VO_2 were increased. Animals treated with iloprost remained baseline levels post-LPS (Fig. 8).

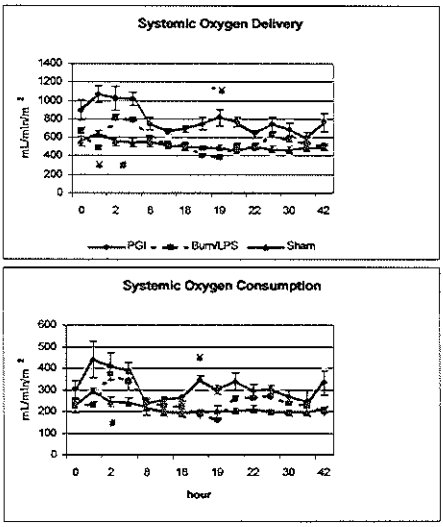

Fig 8. Effects of burn, endotoxin, and postburn administration of iloprost on systemic oxygen delivery (DO_2) and systemic oxygen consumption (VO_2). P <0.05, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

Hepatic Oxygen Delivery and Consumption:

During the first 4 hours postburn, hDO_2 and hVO_2 showed a significant drop to approximately 63% of baseline (Fig. 9). Iloprost treatment did not have any beneficial effects on hDO_2 and hVO_2 after thermal injury. As a matter of fact, both hDO_2 and hVO_2 were reduced to approximately 61% during the entire postburn period (Fig. 9).

Administration of LPS resulted in a significant reduction in hDO_2 to a level of 12% of baseline during the first 2 hours post-LPS and remained as low as 56% of baseline at 4 hours post-LPS (Fig. 9). Postburn treatment with iloprost prevented this impact of LPS, as hDO_2 increased 40% above the postburn level for 2 hours and then remained within baseline range. hVO_2 showed a similar pattern, whereas a significant decrease of 21% of baseline values was calculated during the first 2 hours post-LPS, followed by a 67% increase at 8 hours post-LPS. Animals receiving iloprost remained baseline levels post-LPS (Fig. 9).

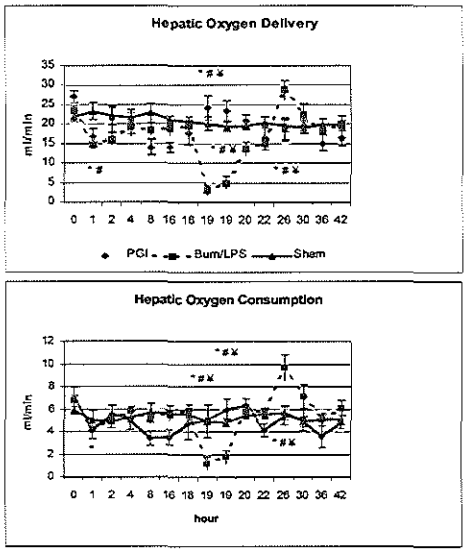

Fig 9. Hepatic oxygen delivery (hDO_2) and hepatic oxygen consumption (hVO_2) showed a significant drop after burn and endotoxin. Iloprost treatment significantly attenuated the impact of only the second insult (LPS). $P < 0.05$, * vs. baseline, # vs. sham, ¥ vs. treatment group (PGI_2).

Discussion

There is compelling experimental evidence 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 post-injury sepsis [12]. As the liver appears to have a gate function for endogenous bacteria and their endotoxins, it appears 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].

Thermal injury appears to induce a selective vasoconstrictive effect on the hepatic arterial circulation, yielding hepatic ischemia and a reduction of hepatic oxygen delivery. The magnitude of the hepatic response to a second insult (endotoxemia) is magnified and manifested as a pronounced hepatic ischemia and reperfusion episode, associated with an inadequate hepatic oxygen delivery and a pathologic supply-dependent hepatic oxygen consumption [5, 6].

A variety of autoregulatory mechanisms are involved in maintaining adequate splanchnic perfusion during periods of low flow [13]. The systemic inflammatory response to severe injuries and/or sepsis encompasses several cellular interactions, leading to the generation of diverse proinflammatory cytokines and vasoactive peptides. The vasoactive metabolite of arachidonic acid, prostacyclin (PGI_2), appears to play a pivotal role in the process of mesenteric ischemia following burn or sepsis. In a 25% TBSA burn model splanchnic vasodilator eicosanoid (PGI_2) release was decreased by 50% postburn. Loss of this endogenous splanchnic vasodilator may contribute to ischemia of the splanchnic vascular bed at this critical time period following acute burn injury [14]. We therefore tested whether prostacyclin treatment can improve hepatic perfusion after burn.

Despite its well-known vasodilating effects, iloprost did not have any significant impact on systemic hemodynamic parameters during the postburn phase. This observation is in agreement with previous studies, showing that the dose that was used in this study has no circulatory effects [15]. Unexpectedly, no improvement was noticed in postburn hepatic perfusion in the iloprost treatment group. While the postburn ischemic phase was manifested for only 4 hours in the untreated animals, HABF was reduced

during the whole postburn period in the treated group. The finding that postburn prostacyclin treatment resulted in a 1.8-fold increase in HAVR shows that prostacyclin did not exert any vasodilating action at all on the hepatic arterial circulation. A possible explanation for this surprising finding is that the dose which we applied in our study was not sufficient enough to overcome the vasoconstrictive impact of thermal trauma on the hepatic arterial circulation. However, similar schemes of administration and dose of iloprost have been used in previous studies. In those studies iloprost was found to effectively improve splanchnic circulation [15-17]. On the other hand, in comparison with the design of our study, the previous experiments were different in three aspects. Iloprost was given in those studies as a pretreatment to examine its effect on intestinal perfusion in sepsis. As iloprost pretreatment was also found to increase liver blood flow in a hyperdynamic model of sepsis [18, 19], one should conclude that iloprost can apparently enhance hepatic perfusion after certain insults such as endotoxemia, providing that the administration of which starts before the insult. For obvious reasons we have chosen in our study to investigate the efficiency of iloprost as a postburn treatment. Little is known about the possible effects of exogenous prostacyclin in the early postburn phase. One study has reported that administration of a prostacyclin analogue did not improve survival after infection in burned mice [20]. The mechanism by which splanchnic blood flow is reduced after thermal injuries involves altered release of arachidonic acid metabolites, resulting in a ratio of vasodilator prostacyclin (PGI_2) and vasoconstrictor thromboxane A_2, favoring the latter [21]. Therefore, it could be argued for that administration of exogenous PGI_2 according to our study protocol, failed in restoring the equilibrium between these vasomediators. Therefore, further studies investigating dose-related effects of PGI_2 analogues and the impact of administration of exogenous PGI_2 on the feedback mechanisms in relation to endogenous prostacyclins and other mediators, such as thromboxanes, are warranted in order to elucidate our results and possible therapeutic potentials of exogenous prostacyclin during the early phase postburn.

Another important finding in our study was the observation of the positive impact of the administration of PGI_2 on hepatic perfusion during the late postburn phase, e.g. after the second insult (LPS). The second insult (LPS) yielded a significant elevation in HAVR (15-fold vs. baseline), which was associated with a critical reduction of HABF to

22% of baseline during the first 4 hours after endotoxin. After recovery to baseline, a reperfusion episode followed with an elevation of HABF to 148% of baseline lasting for a period of 6 hours. On the contrary, HABF and HAVR remained their baseline values during the whole post-LPS phase (24 hours) in burned animals receiving PGI_2 infusion. This beneficial effect of PGI_2 administration as documented in our study is completely in agreement with previous studies investigating the effect of PGI_2 in sepsis. Several experimental studies have reported that PGI_2 can increase splanchnic blood flow in septic animals [7]. In animal studies, exogenous administration of PGI_2 and analogues has been shown to exert beneficial effects on the redistribution of blood flow to the hepatosplanchnic area [16]. Raper et al found that PGI_2 increased liver blood flow in a hyperdynamic model of sepsis [18]. More importantly, Trager et al observed in a hyperdynamic endotoxic pig model that iloprost not only increased liver blood flow but also attenuated the endotoxin-induced metabolic alterations as reflected by a blunted decrease in liver lactate consumption and increase in hepatic venous lactate/pyruvate ratio [19]. However, in contrast with our study, in all these previous studies the effect of PGI_2 administration was solely studied in hyperdynamic endotoxic models. To date, no studies have been conducted to investigate the possible actions of prostacylin treatment on hepatic perfusion during the period of septic complications following thermal injuries. The findings of our study clearly show that postburn prostacylin treatment is beneficial in marinating adequate hepatic perfusion during the late postburn endotoxic phase. The explanation of the paradoxical effects of iloprost during the endotoxic postburn phase in comparison with the early postburn period might be that different pathways or interactions with other mediators are involved in these different processes. Another explanation might be that the effect of prostacyclin on HABF is dose-related under different pathological events such as burn and sepsis. More studies on this area are certainly needed in order to clarify the fundaments of these conflicting data.

Both burn and endotoxin had a striking impact on hepatic oxygenation. A pathologic flow-dependent hVO_2 response was observed, with a pronounced and prolonged hypoxic period. The first 4 hours postburn were marked by a significant decrease in hDO_2 and hVO_2. Administration of LPS to burned animals led to a significant reduction in both hDO_2 and hVO_2 (12% and 21% of baseline, respectively). This early

decreased hVO_2 indicates the inability of the liver to compensate inadequate oxygen delivery by increasing oxygen extraction, resulting in tissue hypoxia. 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 [22]. In agreement with previous data, administration of prostacyclin in our burn/sepsis model was associated with enhanced hepatic oxygen delivery and consumption. In a porcine model of septic shock, iloprost tended to attenuate the reduction in liver oxygen delivery [16]. In a hyperdynamic endotoxic pig model, iloprost was found to significantly attenuate the endotoxin-induced derangements of cellular energy metabolism as reflected by the diminished progressive decrease in hepatic lactate uptake rate [19]. In a porcine model of septic shock, iloprost tended to attenuate the reduction in liver oxygen delivery [16]. The experience with prostacyclin in patients with sepsis is more limited. Lehmann et al. observed that prostacyclin increased the rate of disappearance of indocyanine green, a factor influenced by both liver blood flow and function, indicating improvement in liver function [23]. Recently, in patients with septic shock iloprost was reported to increase splanchnic blood flow and shift oxygen utilization from the energy requiring de novo glucose production rate to other oxygen-demanding metabolic pathways [24]. However, this beneficial effect of iloprost on hepatic oxygenation was only noticed during the late endotoxic postburn phase. In parallel to the effect of prostacyclin on HABF, iloprost failed to improve hepatic oxygenation during the early postburn phase. The consistent effect of prostacyclin on DO_2 and VO_2 after both burn and endotoxin in comparison with its differential impact on hDO_2 and hVO_2 may argue for its selective range of action.

Interestingly was the observation that administration of prostacyclin in this study not only altered HABF but also PVBF. The response of the HABF to burn was unrelated to changes in portal circulation. An early postburn hepatic arterial vasoconstriction was found to occur prior to changes in portal circulation (1 hour vs. 2 hours postburn), demonstrating the relative independence of hepatic arterial response in relation with other splanchnic blood flow. Postburn treatment with iloprost was found to ameliorate to a certain extent the impact of burn on PVBF. The function of PVBF as the major intrinsic regulator of hepatic arterial tone is known as the hepatic arterial buffer response [25]. This

buffer function appears to depend on portal blood flow washing away local concentrations of adenosine from the area of arterial resistance. The similar biphasic pattern of PVBF and HABF after the second insult (LPS) clearly indicates a loss of the hepatic arterial buffer response, as noticed in previous studies [6, 26]. Despite the marked reduction in PVBF during the first 8 hours after endotoxin, HABF showed a significant decrease for a period of 6 hours. On the contrary, PVBF in iloprost-treated animals remained steady within baseline values during the whole period after endotoxin. This observation together with the finding that iloprost had exert the same effects on HABF during the same period demonstrates that iloprost was able to increase the total hepatic blood flow during the late postburn endotoxic phase. The exact pathways by which prostacyclin interferes with the different components of the hepatic circulation, and any possible interaction with other local mediators, remain to be delineated.

The marked portal hypertension seen in the postburn endotoxemic period may account for the occurrence of bacterial translocation and contribute to the previously reported phenomenon of endotoxin-induced bacterial translocation [27]. Portal hypertension initiated by endotoxin has also been shown to induce hepatic microcirculatory disturbance, which may cause liver injury [28]. Administration of prostacyclin to burned animals resulted in reducing PVP and subsequently attenuating portal hypertension during the late postburn endotoxemia. This specific effect of prostacyclin could be of clinical importance, as postburn administration of prostacyclin may reduce the incidence of hepatic injuries and bacterial translocation in the septic phase.

In conclusion, postburn treatment with prostacyclin appears to have specific, but phase-dependent, effects on hepatic perfusion and oxygenation. The results presented in this study suggest that prostacyclin as applied in our model does not have any beneficial effects in preventing hepatic ischemia and reperfusion injury in the early postburn phase. Quite the opposite, postburn prostacyclin treatment was found to significantly enhance hepatic blood flow and oxygen supply during the late postburn septic phase. In addition, postburn prostacyclin administration seems to attenuate burn- and endotoxin-induced portal hypertension. Because of the possible clinical significance of these data, further studies in this area are warranted.

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CHAPTER 10

Regional Inflammatory Response Syndrome "RIRS"

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Submitted to Arch Surg

Abstract

Regional inflammation is normally a process that encompasses various host reactions in response to regional stimuli or in contribution to a systemic response, primarily with a host protective function. However, certain stimuli, such as ischemia, tissue necrosis or uncontrolled infection can alter the balance between the stimulatory and inhibitory mechanisms underlying this process, leading to a hyperactive status that is called the regional inflammatory response syndrome "RIRS". The defective regional cell immune response subsequent to severe injuries, together with the compensatory overgeneration of different immunomodulatory factors, have a detrimental impact on the host susceptibility to infections and cellular repair capacity. The consequent further progression of this response frequently leads to the point of the initiation of its systemic equivalent, the so-called systemic inflammatory response syndrome "SIRS". Therefore, it is proposed that an early and adequate regulation of RIRS would be beneficial not only regionally but also systemically.

Introduction

Our current understanding of sepsis and multiple organ dysfunction syndrome (MODS) needs to be revised, as the uniformly negative results of new therapies for these disorders suggest. Previous theories for the pathogenesis of these conditions are incomplete, as the surrogate models that have been used to study these disorders are not analogous to the clinical situation and patients who have less severe manifestations of these diseases are often overlooked [1]. Most of the conducted experimental and clinical studies are focused on the systemic inflammatory response in the development of time-dependent outcomes that may contribute to sepsis and multiple organ failure (MOF) [2, 3]. Although a considerable effort has been taken in an attempt to define the pathways and mechanisms by which a local injury or an infectious focus eventually transforms into SIRS, there is to date no conceivable mapping of this process. Localized inflammation is a physiological protective response, which is generally tightly controlled by the body at the site of injury. Loss of this local control or an overly activated response is thought to be the initiator of an exaggerated systemic response that is clinically identified as systemic inflammatory response syndrome (SIRS) [4]. Spillover of proinflammatory mediators from the site of injury into the systemic circulation is by now one of the most accepted theories describing the pathogenesis of SIRS [5, 6]. Several studies have investigated these mediators with the result that a considerable number of the puzzle's pieces have been uncovered [7]. However, most of these studies were addressing the local injury as the first step in the cascade towards SIRS or as the precursor of the elements needed to fire up the systemic inflammatory response, all with less appreciation of the role of regional inflammatory response, a fundamental entity in this process.

Therefore, the aim of this paper is to introduce a new concept regarding the post-injury inflammation sequel and to present RIRS as an identifiable stage between local injury and SIRS (Fig.1). In this paper an attempt is made to introduce a conceptual framework for the pathogenesis of MODS, in which local inflammatory response evolves to RIRS and eventually SIRS, which hopefully will contribute to better understanding of the inflammatory response and its sequelae and better approach to effective therapy.

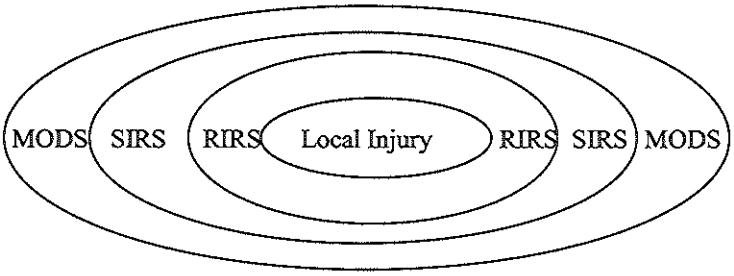

Fig. 1: Conceptual model of RIRS as an exclusive stage in the development of MODS.
RIRS = regional inflammatory response syndrome, SIRS = systemic inflammatory response syndrome, MODS = multiple organ dysfunction syndrome.

Concept of staged inflammation

SIRS is seen in three separate scenarios at present: (1) invasive infection; (2) dissemination of microbes secondary to failure of host defense mechanisms; and (3) severe activation of inflammation by injury, shock, severe soft tissue inflammation, and other noninfectious but proinflammatory events [6]. Obviously, the first two scenarios are more comprehensible and presumably less complicated to manage than the third one. The global hypothesis is that a local injury through various pathways triggers the host inflammatory response, leading to a state of systemic hyperinflammation (i.e. SIRS). Mild to moderate SIRS is most likely beneficial (equal to the normal injury stress response), but severe SIRS can precipitate early MODS, whereas the intensity of SIRS is dependent upon the amount of tissue injury [8]. Thus, in this conceptual construction the relation between the primary local focus and the resultant systemic response is defined in a causal one-step model. Alternatively, a less severe traumatic insult can create an inflammatory environment (i.e. primes the host) such that a later, otherwise innocuous, secondary inflammatory insult precipitates severe SIRS. In this conceptual model, known as the two-hit theory, the interaction between the local and systemic inflammatory response is outlined in a more dynamic two-steps model [9].

Marshall has recently postulated a three-stage development of SIRS, in which stage one is a local production of cytokines in response to an injury or infection. Stage two is the protective release of a small amount of cytokines into the body's circulation. Stage three is the massive systemic reaction where cytokines turn destructive by compromising the integrity of the capillary walls and flooding end organs [10]. In this paper a four-phase concept (Fig. 2) of how local inflammation eventually becomes SIRS is proposed, in which phase one the local inflammatory response to a local stimulus, e.g. injury, infection or hypoxia, triggers an early systemic inflammatory response. In the second phase, both local and systemic mediators initiate a late regional inflammatory response. As a result of an overstimulation of the regional inflammation through ongoing positive feedbacks or inadequate negative feedbacks, a state of hyperactivation of the regional inflammatory response (RIRS) emerges during the third phase. Subsequently, through either insufficient regional host defense or spillover of regional mediators into the circulation and defective feedback pathways RIRS evolves to SIRS.

The four phases of the host inflammatory response to a local stimulus, of which the first two stages are to be considered as the normal physiologic host defense and the following two as the

pathologic exaggerated host reaction, are further delineated.

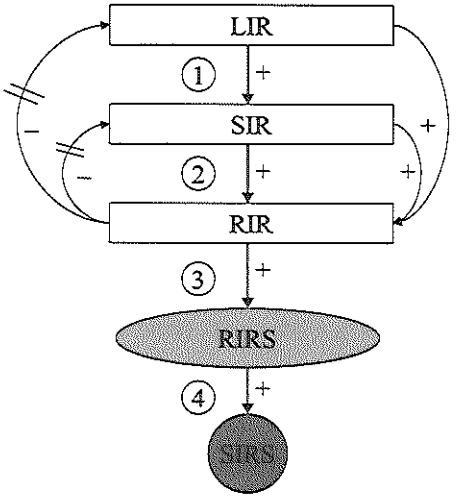

Fig.2: Conceptual model of the spectrum from local inflammation through RIRS to SIRS.
LIR = local inflammatory response, SIR = systemic inflammatory response, RIR = regional inflammatory response, RIRS = regional inflammatory response syndrome, SIRS = systemic inflammatory response syndrome

The first phase: local inflammation to systemic inflammation

Although it is more conceivable that local inflammation is a process that is contained into the site of primary injury or infectious focus, this scenario is obviously valid in case of mild injuries or controlled infections. The host response to such injuries is usually appropriate in degree and is self-limited. In other situations, where severe injuries and/or infections are involved, the need of immediate systemic reinforcement becomes obvious. Under such circumstances, in response to outside aggression, the organism tries to defend itself with two mechanisms: a nonspecific humoral and cellular response called inflammation, and a specific antigenic response that modifies the genetic codes of cells of the defense system and constitutes an immunologic response. Local release of potent inflammatory mediators (cytokines, complement, arachidonic acid derivatives, reactive oxygen metabolites) primarily induces a repair process [11]. Through various messengers arriving from the local site of inflammation into the circulation a typical systemic stress response is initiated. These substances are mediated by a complex array of

neutrophil and macrophage products, afferent neural activity and vascular endothelial activation and are acting as triggers for a neuroendocrinologic, metabolic and immunologic host reaction [12]. The resultant systemic inflammatory response in this phase acts primarily as a supportive mechanism enhancing the local host defense and secondarily as a protective agent, either protecting the host from systemic invasion or preparing the host for an eventual occurrence of such event.

The second phase: local inflammation to regional inflammation

There is evidence that the involvement of the regional immune system in the process of inflammatory response to a local injury or infection is a late event, occurring subsequent to its systemic equivalent. Clinically, severe local injuries or infections are associated with signs of activation of systemic inflammatory response that are normally present early in the course of the disease. During this period, no signs of regional inflammation are usually observed. For instance, an uncomplicated appendicitis or pneumonia is frequently associated with fever and leukocytosis, demonstrating the systemic reaction. However, early surgery on uncomplicated appendicitis or cholecystitis normally reveals only signs of local inflammation, but no signs indicating generalized inflammation of the abdominal region (i.e. peritoneum). Experimental studies on the kinetics of macrophage recruitment to the peritoneum following the induction of acute inflammation have shown an inflammatory influx of neutrophils from the circulation during the first 5 days after the onset of inflammation. Resident peritoneal macrophages were observed not until 7 days after inflammation [13]. Thus, activation of the systemic inflammatory response seems to occur prior to the stimulation of the regional immune system. Both local and systemic inflammation seem to participate through different pathways in the alarming of the regional inflammatory response, which in turn acts as an enhancer of the local host defense and also as a natural boundary for the initial insult, diminishing the systemic stress. The peritoneal response to intra-abdominal infection is an excellent illustrator of such regional response, as the peritoneum is known to execute this specific function in 3 ways: direct absorption of bacteria into the lymphatic circulation, local destruction of bacteria through phagocytosis by either resident macrophages or polymorphonuclear granulocytes attracted to the peritoneal cavity, and localization of the infection in the form of an abscess [14].

The third phase: regional inflammatory response syndrome (RIRS)

Under certain conditions, the regional host response may persist inappropriately, leading to a status of hyperactivation of the regional inflammatory response. In general, after injury if fluid resuscitation is adequate and necrotic tissue is debrided, a hypermetabolic state ensues, directed toward supporting repair of injured tissue. Inflammatory cells are recruited to the site of injury and elaborate cytokines, which stimulate regional immune response and promote repair locally. However, if hypoxic or necrotic tissue and/or uncontrolled infection persist, the ongoing activation of the regional inflammatory response [15], through local and systemic mediators and pathways, eventually results in a regional inflammatory response syndrome (RIRS). This syndrome is characterized by a pathologic status of a constantly unregulated regional inflammation.

Research in recent years has examined the mechanisms underlying cellular host defence on regional level. These studies have established that the resident cells, macrophages and mesothelial cells contribute to the initiation, amplification and resolution of regional inflammation [16-18]. Once the inflammatory response is initiated, recent evidence suggests that mesothelial cells, upon activation by resident macrophages-derived IL-1β and TNF-α, are capable of amplifying inflammation and generating signals (via the creation of a gradient of chemotactic cytokines, IL-8, MCP-1 and RANTES) for the recruitment of leukocytes [19]. This process is also facilitated via the cytokine-driven up-regulation of adhesion molecule expression (ICAM-1 and VCAM- 1) on the mesothelial cells [20]. Much less is understood about the mechanisms by which inflammation is resolved. The secretion of anti-inflammatory molecules (IL-6, IL-1ra and soluble TNF-p55/75) by receptors on resident cells, macrophages and mesothelial cells seems to play an important role in the process [19]. The regional inflammatory state appears also to regulate, through the production of regional substances, the production of eicosanoids in general, and leukotriene C4 (LTC4) and prostacyclin (PGI_2) in particular by the macrophage [21].

The persistence of local or systemic proinflammatory stimuli and/or defective negative feedback pathways may alter the balance between the regional pro- and anti-inflammatory mediators. Consequently, this chaos initiates sequelae of uncontrolled proinflammatory reaction, including microvascular coagulopathy, endothelial activation, increased permeability, transudation, and dysregulated apoptosis [22], all of which maintain the ongoing regional inflammation. An

unbalanced compensatory anti-inflammatory response can result in regional anergy and immunosuppression, resulting in dysfunction of regional organs, such as an ileus, which in turn sets the stage for progressive gut dysfunction with the result that the proximal gut becomes a reservoir for pathogens and toxins that contribute to late infections and amplify inflammatory response [23]. Thus, the regional proinflammatory and anti-inflammatory forces may ultimately reinforce each other, creating a state of increasingly destructive immunologic dissonance [1].

The fourth phase: regional (RIRS) to systemic inflammation (SIRS)

As the regional inflammation continues in an uncontrolled fashion, more biochemical mediators of inflammation are released, with a subsequent spillover into the systemic circulation, triggering remote inflammation [7]. The ongoing regional activation of various humoral (e.g. complement, coagulation) and cellular systems (neutrophils, endothelial cells, macrophages), and the subsequent excessive synthesis, expression and release of numerous mediators (toxic oxygen products, proteolytic enzymes, adherence molecules, cytokines) lead eventually directly, through spillover, or indirectly, through enhancing or suppressing certain systemic inflammatory pathways to a generalized inflammation [24]. Thus, the regional inflammation becomes the precursor of its systemic equivalent. The transformation of acute pancreatitis from a local to regional and then into a systemic inflammation illustrates this phenomenon, as oxygen-derived free radicals, many cytokines (IL-1β, IL-6, IL-8), TNF-α, and platelet activating factor, released by peritoneal cells, have been reported to be principal mediators in this process [25].
Moreover, failure of the regional clearance of the present bacterial load due to defective regional defense mechanisms or an aggressive infection may result in systemic invasion of bacteria and their toxic products, with a subsequent bacteremia and endotoxemia, which in turn potentiates the systemic inflammatory response [26]. The accumulation of regional necrotic tissue, which might be either the cause or the resultant of the RIRS, may sustain a process of excessive presentation of antigens to the systemic immune system [27] through the migration of regional hyperactivated inflammatory macrophages into the lymphatic circulation [28], initiating an autodestructive inflammatory response [2].

Interactions between RIRS and SIRS

Regional inflammation is a physiological protective response that is generally controlled by the body inside the involved region. Loss of this regional control or an excessively activated response (i.e. RIRS) may result in an exaggerated systemic response, which is clinically identified as systemic inflammatory response syndrome (SIRS) [4]. Compensatory mechanisms are initiated in concert with regional and systemic responses and outcome (resolution, multiple organ dysfunction syndrome or death) is dependent on the balance of RIRS and SIRS and such compensatory mechanisms.

SIRS is paradoxically associated with an excessive inflammation [29], as shown by an exacerbated production of cytokines, and an acquired state of immunodeficiency [30], as assessed by a diminished ability of circulating leukocytes to produce cytokine upon in vitro activation. While cytokines are generally viewed as a destructive development in SIRS patients that generally leads to multiple organ dysfunction, cytokines also protect the body when localized [31]. In a review of recent reports, Cavaillon [32] illustrated that the immune-depression reported in SIRS patients, often revealed by a diminished capacity of leukocytes to respond to lipopolysaccharide, is not a generalized phenomenon and that SIRS is associated with a compartmentalized responsiveness, which involves either anergic or primed cells. Also the responses of macrophages appear to vary from location to location and over time in the same organism in response to injury [33]. Thus, it appears that once RIRS evolves to SIRS, a time- and most likely region-dependent hyper- or hypo-activated inflammatory response emerges into a dynamic network of inflammatory actions and reactions.

Conclusion

RIRS is an identifiable stage between tissue injury and the development of SIRS, associated with global inflammatory signs, but also with region specific symptoms. It arises in chaos of responses through the interactions of a network of local, regional and systemic inflammatory responses. The emerging clinical syndrome reflects the state of a regional hyperactivated inflammatory response to a local insult. As the four criteria that define SIRS are nonspecific measures of physiologic severity, rather than distinctive manifestations of a disease process [10], and as SIRS and MODS appear to be considered as various stages of illness progressing to death, which cannot be treated specifically [34], it seems apparent that an early recognition of more

specific stages in these inflammatory cascades is of clinical importance. RIRS is not only a conceptual model, but also provides a clinical relevant description of an early specific event in the inflammatory process, which can be clinically distinguished during the course of an insult to SIRS and ultimately MODS. The understanding of the inflammatory fundamentals of RIRS will most likely facilitate elucidating the more complex pathophysiology of critical illness. As the pathological scope of RIRS seems to be narrower than SIRS and MODS, a target-oriented approach including early control of regional inflammation seems conceptually a more attractive therapeutic option, either as a preventive measure for subjects at risk or as a potential adjuvant treatment for patients with MODS.

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CHAPTER 11

General Discussion

Introduction

Multiple organ failure (MODS) is the cause of 50% to 80% of all deaths in surgical intensive care units [1]. Although MODS was first described more than two decades ago, the cause of the condition is still poorly understood, the treatment is still largely supportive, and the mortality rate (30-70% in most series) is little improved [2]. The clinical picture of MODS is indicative of generalized systemic inflammatory response syndrome (SIRS), which typically occurs as a result of serious infection or severe trauma [3]. SIRS can proceed to MODS as organ systems fail. The response of the host to injury or infection is probably more important in the genesis of SIRS and MODS than the microbial agent or the initiating insult [4]. Thus, an appreciation of the host response in the pathogenesis of MODS is vital if physicians are to develop appropriate modalities for the prevention and treatment of this syndrome.

Two-hit theory of MODS

In many patients, MODS is ascribed to the summation of several insults rather than one event [5]. Each of these insults may be clinically insignificant but may prime the host immune system so that the inflammatory response to subsequent secondary events becomes exaggerated, culminating in SIRS and MODS [4]. This "two-hit" theory may explain how trauma can convert a nonlethal infectious or hypoxic challenge into a lethal insult (chapter 1). In an attempt to mimic the clinical situation, which is commonly complicated by sepsis and endotoxin release, we utilized in the experimental studies of this thesis a combined burn and endotoxin porcine model. The second insult "endotoxemia" clearly amplified the host response and consequently resulted in augmenting the adverse impact of the initial insult "trauma".

Splanchnic ischemia and reperfusion injury

A consistent temporal association between shock and the later development of MODS indicates that shock is an important etiologic factor in the pathogenesis of MODS [6]. Adequate resuscitation and oxygenation as global measures in restoring and maintaining optimal organ perfusion are considered to be life saving procedures in the early phase of

managing severely injured patients. However, clinical studies using aggressive volume resuscitation and supranormal oxygen delivery strategies have failed to show a reduction in the incidence of MODS or a survival benefit in patients developing SIRS and MODS [7, 4]. Laboratory observations have shown that shock induces disproportionate splanchnic hypoperfusion [8]. In the clinical setting, several investigatory groups have demonstrated that gut hypoperfusion, despite maximal resuscitative efforts, is predictive for MODS and death [9, 10]. Thus, splanchnic ischemia has been implicated as an important inciting event in the pathogenesis of MODS. In our animal studies (chapters 4 and 6), severe trauma (40% TBSA third-degree burn) resulted in a significant decrease (approximately 58%) in mesenteric blood flow. This occurred despite a resuscitation format that resulted in normalization of cardiac output and systemic oxygen delivery. The importance of these findings is that maintenance of systemic hemodynamic parameters does not necessarily result in normal mesenteric blood flow. The second insult (endotoxemia) had a more pronounced and prolonged impact on mesenteric circulation and oxygenation, demonstrating the priming effect of the initial injury.

The splanchnic ischemic period following trauma and in sepsis, as documented in our porcine model (chapters 4 and 6), was consistently followed by a mesenteric reperfusion phase. Reperfusion intestinal tissue damage appears to be due to the formation of oxygen radicals and the activation of phospholipase A_2. The initial source of oxygen radicals seems to be the hypoxanthine-xanthine oxidase system. Oxygen radicals react directly with poly-unsaturated fatty acids, leading to lipid peroxidation within the cell membranes. Indirectly, the radicals trigger the accumulation of neutrophils within the affected tissue initiating inflammatory processes that lead to severe mucosal lesions [11]. Lipid peroxidation, as indicated in our model by plasma conjugated dienes concentrations, showed a 68% and 210% increase after burn and in sepsis, respectively (chapter 5).

Intestinal permeability

The manifestation of mesenteric ischemia and reperfusion injury included a marked increase in intestinal permeability, measured using the lactulose/mannitol ratio (chapter 5 and 6). The linear correlation, observed in our model, between both elevated plasma

conjugated dienes and increased permeability index suggests a causal relationship (chapter 5). Clinical implications of altered intestinal permeability were documented by Levoyer et al [12], who noted that patients with the most sustained and marked alterations in intestinal permeability were at increased risk for subsequent systemic infections. The hypothesis that dysfunction of the intestinal mucosal barrier contributes to the development of MODS is supported by clinical evidence that intestinal permeability is increased in patients developing sepsis [13] after major thermal injury [14].

Bacterial translocation

The hypothesis that gut-derived bacteria or their products contribute to the development of MODS has been introduced to explain why no identifiable focus of infection can be found in as many as 30% of bacteremic patients who die from MODS [4]. Several investigators have demonstrated that hemorrhagic shock causes bacterial translocation to mesenteric lymph nodes, liver, spleen and systemic circulation [15, 16]. In our model, bacterial translocation to mesenteric lymph nodes and remote organs occurred in almost all animals receiving thermal injury and endotoxin (chapters 5 and 6). The clinical relevance of altered gut barrier function was challenged in a human study by Moore et al [17], in which they evaluated bacterial translocation after trauma. In this prospective clinical study, the investigators failed to identify any significant concentration of endotoxin or bacteria in portal venous blood and therefore concluded that the occurrence of bacterial translocation in humans is uncertain. A possible explanation for this apparent controversy is that there are other conduits such as gut-derived mesenteric lymph through which gut-derived factors may reach the systemic circulation [18]. These investigators have demonstrated that after hemorrhagic shock, mesenteric lymph induces endothelial injury and contributes to lung injury. Furthermore, mesenteric lymphatic division before thermal injury was found to prevent acute lung injury, supporting the hypothesis that gut-derived factors carried in mesenteric lymph contribute to trauma-induced remote organ injury [19]. Consequently, the gut hypothesis of MODS has been expanded to include gut-derived inflammatory factors and bacteria.

Hepatic ischemia and reperfusion injury

Acute hepatic dysfunction is a well-recognized component of MODS. Hepatic ischemia and reperfusion have been documented to activate Kupffer cells and increase cytokine levels, which may produce systemic inflammation and may be responsible for tissue injury locally and on remote sites [22]. Although hepatic ischemia and reperfusion have been extensively investigated in transplantation studies, little is known about the impact of severe injuries and sepsis on hepatic circulation and oxygenation. The results of our experimental studies (chapters 7, 8 and 9) demonstrated that trauma induces a selective vasoconstrictive effect on the hepatic circulation, yielding hepatic ischemia and a reduction of hepatic oxygen delivery. The magnitude of the hepatic response to a second insult (sepsis) is magnified and manifested as a pronounced hepatic ischemia and reperfusion episode, associated with an inadequate hepatic oxygen delivery and pathologic supply-dependent hepatic oxygen consumption. These alterations in hepatic hemodynamics and oxygenation were independent of the systemic parameters, indicating the involvment of specific pathways.

There is a large body of evidence that the liver microcirculation has to be considered as a major target in hepatic ischemia and reperfusion injury. The nature of microvascular injury, which precedes manifestation of hepatic parenchymal tissue damage, includes both hypoxia due to lack of microvascular perfusion (i.e. no-reflow), and a reperfusion-associated inflammatory response, which includes the activation and dysfunction of leukocytes and Kupffer cells (the reflow paradox). No-reflow in sinusoids is thought to be caused by endothelial cell swelling and intravascular hemoconcentration, and involves also a deterioration of the balance between endothelins (ET) and nitric oxide (NO). The reflow paradox is associated with: the release and action of proinflammatory cytokines (TNF-alpha, IL-1) and oxygen radicals, the up-regulation of endothelial and leukocytic adhesion molecules (selectins, beta-integrins, ICAM-1), and the interaction of leukocytes with the endothelial lining of the hepatic microvasculature [21].

As the liver appears to have a gate function for gut-derived bacteria and their products, it appears that impairment of this hepatic clearance function may potentiate systemic effects of gut barrier failure by allowing indigenous bacteria or their endotoxins to reach the systemic circulation (chapter 3), where they potentiate systemic inflammatory

responses [5]. Furthermore, when translocated bacteria and endotoxin reach the liver, activation of Kupffer cells occurs, resulting in the secretion of proinflammatory mediators that enhance the stress response and alter various immune functions [23]. These observations in conjunction with our previous data on intestinal permeability and bacterial translocation may support the hypothesis of the gut-liver axis in the pathogenesis of MODS, whereas the gut is the "starter" of MODS and the liver is the "motor" of MODS [24]. The associated increased portal venous resistance and portal hypertension documented in our model (chapters 7, 8 and 9) may also contribute to the occurrence of intestinal mucosal damage and subsequent bacterial or endotoxin translocation (chapter 3).

Splanchnic and hepatic ischemia and reperfusion injury as a regional inflammatory response syndrome "RIRS" model

Although the potential role of bacteria/endotoxin translocation and its clinical relevance remains controversial, many lines of evidence support the concept that early gut hypoperfusion sets the stage for progressive gut dysfunction, such that the gut becomes a reservoir for pathogens and toxins that contribute to SIRS and MODS [16].

In this thesis a four-phase concept of how local inflammation eventually becomes SIRS is introduced. As a result of an overstimulation of the regional inflammation through ongoing positive feedbacks or inadequate negative feedbacks, a state of hyperactivation of the regional inflammatory response (RIRS) emerges. This syndrome is characterized by a pathologic status of a constantly uncontrolled proinflammatory reaction and unbalanced compensatory anti-inflammatory response. Subsequently, through either insufficient regional host defense or spillover of regional mediators into the circulation and defective feedback pathways RIRS evolves to SIRS.

In the four-phase concept of the pathogenesis of post-injury SIRS and ultimately MODS (chapter 10), splanchnic and hepatic ischemia and reperfusion injury can be identified as a precursor of RIRS. The initial regional hypoperfusion induced by the first insult can lead to gut barrier failure, microcirculatory injury and macrophage activation [4]. The ongoing bacterial and endotoxin translocation together with the primed Kupffer cells and

diminished hepatic bacterial clearance capacity activate regional neutrophils sequestration, phospholipase A_2 release [16], synthesis and action of proinflammatory cytokines [20] and oxygen radicals [21], setting up a vicious cycle of uncontrolled regional events (RIRS), leading to a subsequent spillover into the systemic circulation and ultimately culminating in MODS.

Mediators and modulators

The differentiated impact of trauma and sepsis on splanchnic and hepatic perfusion and oxygenation, independently of systemic parameters, together with the importance of the induced regional ischemia and reperfusion injury in the pathogenesis of SIRS and MODS necessitate a target-oriented approach including early augmentation of intestinal and hepatic perfusion and oxygenation.

The systemic inflammatory response to severe injuries and/or sepsis encompasses several cellular interactions, leading to the generation of diverse proinflammatory cytokines and vasoactive peptides. Some of these substances appear to play a pivotal role in the process of mesenteric ischemia following burn or sepsis (chapter 2).

The renin-angiotensin axis appears to play a critical role in the pathophysiology of post-injury splanchnic and hepatic ischemia. Angiotensin II, a potent vasoconstrictor that exhibits important mesenteric selectivity, has been identified in our model as an important mediator in the process by which trauma and sepsis adversely affect splanchnic (chapter 4) and hepatic (chapter 8) perfusion and oxygenation. The results of our experiments clearly show that blocking the action of angiotensin II using a specific angiotensin II receptor antagonist can ameliorate trauma- and sepsis-induced mesenteric and hepatic ischemia by improving regional blood flow and oxygen supply (chapters 4 and 8). The same treatment modality has also been shown to reduce intestinal permeability and to prevent bacterial translocation after severe trauma and in sepsis (chapter 5).

As interleukin-1α (IL-1α) has been reported to affect receptors for the peptide hormone angiotensin II [25], we studied the effects of post-injury IL-1α treatment in our model. Our data clearly show that administration of IL-1α attenuates mesenteric ischemia and reperfusion injury induced by trauma and endotoxemia (chapter 6). This intervention

strategy also yielded a significant reduction in intestinal permeability and bacterial translocation (chapter 6).

As IL-1 has been documented to cause an increase in the vasodilator prostacyclin concentrations [26], we also studied the effects of prostacyclin in our model. According to the data collected in our studies (chapter 9), treatment with prostacyclin appears to have specific, but phase-dependent, effects on hepatic perfusion and oxygenation. Our findings suggest that prostacyclin, as applied in our model, does not have any beneficial effects in preventing hepatic ischemia and reperfusion injury in the early post-injury phase. Quite the opposite, administration of prostacyclin significantly enhances hepatic blood flow and oxygen supply during the late post-injury septic phase.

Thus, the results of our studies clearly show that interventional strategies regionally directed towards an early enhancement of mesenteric and hepatic perfusion and oxygenation, through target-specific pathways, are effective in attenuating splanchnic and hepatic ischemia and reperfusion injury induced by trauma and sepsis. The possible clinical significance of these findings and the potential benefits of these interventional modalities in preventing MODS and improving the outcomes of severely injured and septic patients warrant further research in this field.

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CHAPTER 12

Summary and Conclusions

Chapter 1

In the introduction of this thesis a brief overview is given of multiple organ dysfunction syndrome (MODS). It emphasizes the conceptual models describing the pathophysiological changes in the process of development of MODS in the surgical intensive care unit. Interventional strategies to prevent or treat this process and their benefits and limitations are also discussed. The background of the experimental studies included in this thesis is expressed in a conceptual model emphasizing the importance of intestinal and hepatic ischemia and reperfusion injuries as early events in the process of post-injury MODS. The studies are designed in an attempt to improve the understanding of the mechanisms through which sequential insults, e.g. injury and sepsis, adversely influence the gut-liver axis and to examine interventional strategies targeting some of the mediators that play a pivotal role in these early events.

Chapter 2

The impact of major trauma, such as severe thermal injury, on intestinal blood flow is delineated in this chapter, with emphasis on the subsequent intestinal ischemia that leads to the translocation of indigenous bacteria and endotoxin to remote body organs. The synergic impact of sepsis as a second insult on the primed host, in amplifying trauma-induced intestinal ischemia and reperfusion injury is outlined. Possible mediators that initiate and maintain this process are reviewed. Interventional strategies interacting with these mediators are discussed.

Chapter 3

The concept of the gut acting as the primary motor of MODS has been getting a lot of scientific attention and most of the investigations were focused on describing, understanding, and eventually influencing this process. In this chapter another concept in the pathophysiology of MODS is introduced. The liver appears to be very susceptible to post-injury ischemic insults. This global hepatic ischemia is implicated in priming the hepatic Kupffer cells yielding a release of several inflammatory mediators and a depression of the hepatic bacterial clearance function, with a subsequent systemic

bacterial and endotoxin spillover. Therefore, the description of the gut as the starter and the liver as the motor for MODS seems more conceivable.

Chapter 4

In a combined burn and sepsis chronic porcine model, a profound impact of both insults on intestinal perfusion and oxygenation is documented. The initial injury causes a significant increase in mesenteric vascular resistance and a subsequent reduction in mesenteric blood flow and oxygen delivery. The impact of the second insult is more pronounced. The study examines the hypothesis that early blockade of angiotensin II receptors can be beneficial in this process. Postburn treatment with DuP753, a specific angiotensin II receptor antagonist, is shown to attenuate the adverse impact of thermal injury and sepsis on intestinal perfusion and oxygenation.

Chapter 5

Increased intestinal permeability after trauma and in sepsis is most likely the resultant of the induced intestinal ischemia and reperfusion injury. Oxygen-derived free radicals have been implicated in the subsequent tissue damage that results in dysfunction of intestinal mucosal barrier. The data of this study clearly show that thermal injury and endotoxemia significantly 1) increase intestinal permeability, as assessed by the lactulose/mannitol excretion ratio, 2) induce reperfusion injury, yielding lipid peroxidation, as demonstrated by the elevation of plasma conjugated dienes, 3) enhance bacterial translocation to remote organs, as shown in increased rates of positive tissue cultures. The results of this study indicate that angiotensin II plays a pivotal role in this process. Blocking angiotensin II receptor by the administration of DuP753 is found to attenuate these detrimental effects of burn and endotoxin on intestinal mucosal integrity, the result of which is a decrease in intestinal permeability and bacterial translocation.

Chapter 6

In this experimental study the effects of administration of interleukin 1α (IL-1α) as a post-burn treatment modality are investigated in a burn and sepsis chronic porcine model. The data of this study show that the cytokine IL-1α plays an important role in intestinal

perfusion and oxygenation after burn and in sepsis. Under such conditions, IL-1α significantly improves mesenteric blood flow and enhances mesenteric oxygen delivery. Subsequently, both intestinal permeability and incidence of bacterial translocation are reduced after the treatment with IL-1α.

Chapter 7

This study is designed to define the hemodynamic response and the tissue perfusion of the liver to severe injuries and sepsis in a combined burn and endotoxin chronic porcine model. Thermal injury has a selective vasoconstrictive impact on hepatic arterial blood flow, yielding hepatic ischemia and a reduction of hepatic oxygen delivery. The response of the primed liver to a second insult, i.e. endotoxemia, is amplified and manifested as a pronounced hepatic ischemia and reperfusion episode, associated with an inadequate hepatic oxygen delivery and a pathologic supply-dependent hepatic oxygen consumption. A loss of the hepatic arterial buffer response, as indicated by a profound decline in both portal venous and hepatic arterial blood flow, seems to be one of the mechanisms responsible for these phenomena. Post-injury sepsis appears to induce severe portal hypertension, which may contribute to gut barrier dysfunction.

Chapter 8

This study examines the hypothesis that angiotensin II, a potent vasoconstrictor, is involved in the occurrence of hepatic ischemia after trauma and in sepsis. Postburn administration of angiotensin II receptor blocker DuP753 significantly improves hepatic arterial hemodynamics. Endotoxemia is documented to cause a significant hepatic arterial vasoconstriction, with a significant increase in both hepatic arterial and portal venous vascular resistance. The administration of angiotensin II receptor antagonist DuP753 significantly enhances hepatic blood flow and oxygen supply. The action of DuP753 appears to be selective. The enhancement in oxygen supply, seen after DuP753 treatment, to meet the increased oxygen demand is only observed in the hepatic circulation and no systemic changes are found. Another beneficial effect of angiotensin II receptor blocking treatment is the prevention of the sepsis-induced portal hypertension. Therefore, it is

concluded that angiotensin II plays a pivotal role in the process of hepatic ischemia and reperfusion injury induced by trauma and endotoxemia.

Chapter 9

The hypothesis that the stable prostacyclin analogue iloprost beneficially influences post-injury hepatic hemodynamics and thereby improves tissue perfusion and oxygenation is investigated in a burn and sepsis porcine model. Postburn treatment with prostacyclin appears to have specific, but phase-dependent, effects on hepatic perfusion and oxygenation. During the early post-injury phase, prostacyclin seems to have no beneficial effects on hepatic perfusion. However, during the late post-injury septic period, prostacyclin seems to significantly improve hepatic total blood flow and oxygen supply. Portal hypertension, induced by thermal injury and sepsis, can be attenuated by prostacyclin treatment.

Chapter 10

The aim of this paper is to introduce a new concept regarding the post-traumatic inflammation sequel. The regional inflammatory response syndrome "RIRS" is presented as an identifiable stage between local injury and the systemic inflammatory response syndrome or "SIRS". The pathophysiology of the process during which RIRS emerges is extensively delineated. Various cellular interactions, biochemical pathways, immunomodulators, and proinflammatory agents that are involved in this process are discussed. Examples of clinical situations that may initiate the manifestation of RIRS are reviewed. The paper also emphasizes the importance of understanding RIRS in providing new insights into the complex process of sequelae of systemic inflammation (SIRS), embodied in the concept of the multiple organ dysfunction syndrome (MODS).

Chapter 11

A general discussion of the thesis is presented in this chapter. The outcomes of our studies are reviewed in one unifying concept. Furthermore, the validity of the conclusions included in this thesis and their clinical implications are debated.

Conclusions

1- Ischemia of the hepato-splanchnic region appears to be an early specific event in the cascade following major trauma and in sepsis.

2- A post-injury septic challenge acts synergistically with the initial injury, amplifying the selective vasconstrictive impact of trauma on hepato-splanchnic blood flow with a subsequent critical tissue hypoxia followed by a profound reperfusion injury.

3- Neither adequate resuscitation nor maintenance of normal cardiac output and systemic oxygen delivery necessarily results in normal hepato-splanchnic perfusion and oxygenation, under such conditions.

4- Manifestations of hepato-splanchnic ischemia and reperfusion injury include lipid peroxidation of cell membranes, mucosal damage, increased permeability and an altered hepatic bacterial clearance function.

5- Alterations in hepato-splanchnic blood flow and oxygenation have potential clinical implications in that hepato-splanchnic barrier function (gut-hepatic axis) is altered, promoting systemic spillover of regional bacteria, endotoxin, and inflammatory mediators.

6- These events are at least partially regulated by angiotensin II, IL-1α and prostacyclin.

7- Administration of a specific angiotensin II inhibitor, IL-1α or prostacyclin attenuates trauma- and sepsis-induced hepato-splanchnic ischemia and reperfusion injury and reduces the subsequent adverse events.

8- RIRS is an identifiable stage between tissue injury and the development of SIRS, associated with global inflammatory signs, but also with region specific symptoms.

9- In the four-phase concept of the pathogenesis of post-injury SIRS and ultimately MODS, hepato-splanchnic ischemia and reperfusion injury can be identified as a precursor of RIRS.

10- The selective impact of trauma and sepsis on hepato-splanchnic perfusion, together with the importance of the induced regional ischemia and reperfusion injury in the pathogenesis of SIRS and MODS, necessitate a target-oriented approach, including early augmentation of hepato-splanchnic perfusion and control of regional inflammation.

Appendices

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