Liver Perfusion in Sepsis, Septic Shock, and Multiorgan Failure

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ABSTRACT
Sepsis causes significant alterations in the hepatic macro- and microcirculation. Diverging views exist on global hepatic blood flow during experimental sepsis because of the large variety in animal and sepsis models. Fluid-resuscitated clinical sepsis is characterized by ongoing liver ischemia due to a defective oxygen extraction despite enhanced perfusion. The effects of vasoactive agents on the hepatosplanchnic circulation are variable, mostly anecdotal, and depend on baseline perfusion, time of drug administration, and use of concomitant medication. Microvascular blood flow disturbances are thought to play a pivotal role in the development of sepsis-induced multiorgan failure. Redistribution of intrahepatic blood flow in concert with a complex interplay between sinusoidal endothelial cells, liver macrophages, and passing leukocytes lead to a decreased perfusion and blood flow velocity in the liver sinusoids. Activation and dysfunction of the endothelial cell barrier with subsequent invasion of neutrophils and formation of microthrombi further enhance liver tissue ischemia and damage. Substances that regulate (micro)vascular tone, such as nitric oxide, endothelin-1, and carbon monoxide, are highly active during sepsis. Possible interactions between these mediators are not well understood, and their therapeutic manipulation produces equivocal or disappointing results. Whether and how standard resuscitation therapy influences the hepatic microvascular response to sepsis is unknown. Indirect evidence supports the concept that improving the microcirculation may prevent or ameliorate sepsis-induced organ failure. Anat Rec, 291:714–720, 2008.

Key words: sepsis; septic shock, organ failure; liver perfusion; microcirculation

Severe sepsis, septic shock, and multiorgan failure are among the most common causes of morbidity and mortality in intensive care units (Angus et al., 2001). Although the septic process starts with the proliferation of microorganisms at an infectious nidus, its evolution is largely determined by a complex systemic and microvascular framework of inflammatory and coagulation reactions that profoundly affect tissue perfusion and oxygenation (Esmon et al., 1999).

The liver is thought to substantially influence metabolic and host defense mechanisms during sepsis. The liver actively modulates inflammatory processes by filtering, inactivating, and clearing bacteria, bacterial products (e.g., endotoxin), vasoactive substances, and inflammatory mediators. In addition, the stimulated liver itself produces and releases high amounts of various cytokines, bioactive lipids, and acute phase proteins (Dhainaut et al., 2001). “Early” hepatic dysfunction occurs in the first hours of sepsis and is related to hepatosplanchnic hypoperfusion. This insult may cause an acute increase in biological markers of liver damage (transaminases, lactate dehydrogenase, bilirubin). However, it is usually rapidly reversed with adequate supportive treatment. In contrast, “late” hepatic dysfunction is a more insidious and ominous process. It is character-
LIVER PERFUSION IN SEPSIS

Assessment of Global Liver Perfusion

Liver perfusion cannot be judged by looking exclusively at systemic hemodynamics. Unfortunately, hepatic circulation is difficult to determine in clinical practice. Rather, measurements are taken from the hepatospalanchnic region as a whole. Total hepatospalanchnic blood flow can be estimated from plasma concentrations of substances that either are cleared exclusively by the liver [e.g., indocyanine green (ICG) dye] or result from liver-specific metabolic pathways [e.g., the lidocaine deethylation product monethylglycinexylidide (MEGX)] (Brinkmann et al., 1998). Some small studies in experimental sepsis (Kaminski et al., 2004) and human septic shock (Kimura et al., 2001) suggest that the elimination rate of ICG can reliably identify liver injury. Assessment of liver blood flow with ICG requires the placement of a hepatic venous catheter, which is technically difficult to perform at the bedside (Uusaro et al., 1995). A device for transcutaneous measurement of ICG plasma clearance (LiMon™, Pulsion Medical Systems) has not been tested in severe clinical sepsis. The MEGX extraction rate can be very variable and unpredictable in the critically ill and may markedly differ from other hepatic metabolic pathways due to intrahepatic metabolic compartmentation (Reinelt et al., 1999).

EXPERIMENTAL STUDIES

Building an overall picture of global hepatic blood flow during sepsis is difficult due to the variety of animal (rodents, dogs, pigs, and sheep) and sepsis (IV bolus or continuous infusion of endotoxin, live bacteria injection, cecal ligation and puncture, and so on) models. Other caveats are the type of anesthesia, the duration of observation, and whether or not the animals are fluid resuscitated. Also, different techniques are used for measuring portal vein and hepatic artery flow. Nevertheless, major findings include a direct correlation between total hepatic blood flow and cardiac output (Pastor et al., 1995a), a decrease of portal blood flow due to a fall of splanchnic flow upon mesenteric artery constriction (Navaratnam et al., 1990), and a defective hepatic arterial buffer response (Ayuse et al., 1995).

CLINICAL STUDIES

Only few studies have examined hepatic perfusion in human sepsis. Hepatospalanchnic blood flow was found to be highly increased in volunteers injected with endotoxin (Fong et al., 1990) and in fluid-resuscitated septic patients (Dahn et al., 1990a). Despite enhanced perfusion and low hepatic venous oxygen saturation, hepatic tissue still appeared to be ischemic. This is explained by a high splanchic oxygen demand and a disproportionate oxygen consumption due to hepatic hypermetabolism that are insufficiently compensated by an increase in blood oxygen extraction (Dahn et al., 1990b).

EFFECT OF TREATMENT ON GLOBAL (HEPATO)SPLANCHNIC PERFUSION IN SEPSIS AND SEPTIC SHOCK

Resuscitation of patients with early severe sepsis and septic shock requires infection control, oxygenation, ample fluid administration, and eventually transfusion and use of vasoactive drugs. The latter aim at adequate blood pressure (mainly vasopressors) and/or effective cardiac output (inotropes, mainly dobutamine) (Marik and Varon, 1998). A protocol-driven implementation of such treatment has been shown to result in less organ failure and a higher survival (Rivers et al., 2001). Its impact on hepatic dysfunction, however, has not been evaluated. Some studies have looked at the effect of vasoactive drugs on regional splanchnic perfusion. In clinical septic shock necessitating vasopressor treatment, cardiac output was higher but splanchnic blood flow lower with epinephrine than with norepinephrine (De Backer et al., 2003). Dopamine also increases mesenteric blood flow, but at high doses this may be associated with a negative hepatic energy balance (Guerin et al., 2005). Vasopressin and its long-acting analogue terlipressin can restore blood pressure in catecholamine-resistant clinical septic shock. Interestingly, vasopressin (van Haren et al., 2003) but not terlipressin (Asfar et al., 2005) was shown to have detrimental effects on hepatospalanchnic perfusion. Dobutamine usually increases splanchnic perfusion in sepsis (Gutierrez et al., 1994; Creteur et al., 1999) but not in septic shock (Reinelt et al., 1997) where its effects are more variable (Doly et al., 1999) and dependent on the adequacy of splanchnic perfusion at baseline (De Backer et al., 1998). Dobutamine does improve regional oxygen availability in canine endotoxic shock (De Backer et al., 1996) and in human sepsis (Gutierrez et al., 1994) and compensates for the deleterious splanchnic hemodynamic effects of vasopressin in porcine endotoxic shock (Martikainen et al., 2004). However, in porcine sepsis, no relationship was found between dobutamine-induced changes in oxygen delivery and the degree of protection of the hepatic ultrastructure (Tighe et al., 1995). Moreover, hepatic morphology was influenced by the type of adrenergic agent rather than by its inotropic potency, with dobutamine eliciting the most devastating effect on hepatic ultrastructure (Tighe et al., 1995). The available clinically relevant data on the effects of doxapamine (a doxapamine analogue with vasodilating effects) (Smithies et al., 1994), enoximone (a phosphodiesterase III inhibitor) (Kern et al., 2001), iloprost (a prostacyclin analogue) (Lehmann et al., 2000), and nitroglycerin (Spronk et al., 2002) is anecdotal and their safety has not been established. N-acetylcysteine (NAC) is a glutathione analogue with antioxidant and, to a lesser extent, anti-inflammatory and vasodilating capacities. In early clinical septic shock, high intravenous doses of NAC increased cardiac output and liver blood flow (Rank et al., 2000). NAC also enhanced liver function as determined by increased MEGX serum concentrations (Rank et al., 2000; Hein...
et al., 2004) and decreased tissue lactate signal intensity (Hein et al., 2004). However, the fractional liver blood flow index (ratio of liver blood flow index/cardiac index) remained unchanged (Rank et al., 2000). This is in agreement with earlier findings in experimental endotoxic shock, showing that NAC increased total but not fractional mesenteric blood flow (Zhang et al., 1995). These observed beneficial effects of NAC on the splanchnic circulation in sepsis must be weighed against potential negative effects of the drug. Indeed, NAC has been reported to significantly depress cardiac performance in late septic shock (Peake et al., 1996) and to worsen outcome if given in established organ failure (Molnar et al., 1999).

**MICROCIRCULATORY HEPATIC BLOOD FLOW DURING SEPSIS**

**Experimental Studies**

Alterations in microvascular blood flow are increasingly incriminated as an important cause of sepsis-induced multiorgan failure. The mechanisms of this microvascular failure are protein and include a reduction in functional capillary density and red blood cell deformability, increased permeability and apoptosis of endothelial cells (ECs), an altered vasomotor tone with opening of arteriovenous shunts, adherence of platelets and activated neutrophils to the EC layer due to increased expression of adhesion molecules at the surface of ECs, and activation of the clotting cascade resulting in fibrin deposition and formation of microthrombi (Spronk et al., 2004). All these processes act together to cause generalized tissue dysoxia, either from impaired microcirculatory oxygen delivery and/or from mitochondrial dysfunction. Clinically, this is perceived as an oxygen extraction deficit.

Sepsis causes severe changes in the microcirculatory blood flow of all splanchnic organs that cannot be predicted from changes in systemic or regional flow. This was elegantly demonstrated in a porcine fecal peritonitis model producing septic shock (Hiltebrand et al., 2000). During shock, systemic, superior mesenteric artery, and microcirculatory liver flow all decreased by approximately 50%. Fluid resuscitation resulted in a threefold increase in systemic and mesenteric flow but only marginally increased microvascular liver perfusion.

Intrahepatic blood flow redistribution is channeling blood away from contracted to dilated vessels, creating a net decrease in perfused sinusoidal area (Unger et al., 1989). Sinusoidal ECs and Kupffer cells (KCs) form the first line contact cells for bacteria, bacterial products (e.g., endotoxin), and microbial debris delivered by the portal and hepatic artery blood. The response of the hepatic microvascular “sieve” upon these substances correlates with the state of activation, number, and distribution of KCs within the hepatic lobuli (Gregory and Wing, 2002; Keller et al., 2005). Upon stimulation, KCs release a host of inflammatory, toxic, and vasoactive mediators that may all directly or indirectly induce tissue injury (Table 1). Sinusoidal ECs produce vasoactive substances [e.g., prostacyclin and nitric oxide (NO)] that modulate vascular tone both in the hepatic macro- and microcirculation. On the other hand, sinusoidal ECs undergo significant structural and functional changes. Swelling, dis-tension, and disruption of sinusoidal ECs cause leakage of albumin, plasma, and inflammatory cells into the interstitium, resulting in direct tissue damage. Importantly, activated sinusoidal ECs lose their normal anti-coagulant state and express surface adhesion molecules attracting massive amounts of platelets and leukocytes (Croner et al., 2006). In the end, sinusoids become plugged with fibrin clots and clusters of blood cells (Fig. 1). The resulting fall in sinusoidal flow velocity adds to the already decreased sinusoidal perfusion area. In the presence of unopposed inflammation and ongoing coagulopathy, this will eventually culminate in microvascular ischemia and dysfunction and finally induce hepatocellular injury and failure.

**TABLE 1. Biologically Active Products of Stimulated Kupffer Cells**

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>IL-1, 6, 8, 12</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine inhibitor</td>
<td>Transforming growth factor β</td>
<td>Interferon α/β</td>
</tr>
<tr>
<td>Complement components</td>
<td>IL-1 receptor antagonist</td>
<td>Prostaglandin D, E₂</td>
</tr>
<tr>
<td>Bioactive lipids</td>
<td>Thromboxane D₃, E₂</td>
<td>NO2, NO3</td>
</tr>
<tr>
<td>Reactive oxygen and nitrogen intermediates</td>
<td>IL = interleukin; TNF = tumor necrosis factor; PAF = platelet activating factor.</td>
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</tr>
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**SUBSTANCES REGULATING HEPATIC MICROVASCULARIZATION AND THEIR ROLE IN SEPSIS**

NO is thought to play a pivotal role in the regulation of hepatic microvascular perfusion. This gaseous molecule is synthesized by NO synthase (NOS). The major NOS isoforms—endothelial (“constitutive”) NOS (eNOS) and inducible NOS (iNOS)—are found in the liver. Under normal conditions, eNOS produces small amounts of NO in response to receptor mediated and shear stress vasodilator stimuli (Boegehold, 1996; Macedo and Lautt, 1996). NO derived from eNOS appears to ensure the integrity of liver microvascular blood flow through vasodilating and platelet antiaggregating effects, inhibition of leukocyte—ECs interactions, regulation of red blood cell rheology, microbial elimination, and neutralization of the highly aggressive superoxide anion (Gundersen et al., 1997; Peralta et al., 2001). During evolving sepsis, however, iNOS becomes up-regulated in hepatocytes, KCs, and sinusoidal ECs as demonstrated by increased levels of the NO metabolites NO₂ and NO₃ in plasma (Shieh et al., 2000) and in liver tissue (Preiser et al., 2001). Excessive and uncontrolled NO release during endotoxemia is held responsible for generalized vasoplegia, hypotension, cardiac depression, and hyporeactivity to adrenergic agents (Cobb and Danner, 1996). Nonsel ective NOS inhibition, however, had detrimental effects on global hepatic perfusion, oxygen transport, and liver morphology (Billiar et al., 1990; Pastor and Payen, 1994) and caused more severe inflammation and sinusoidal blood flow reduction in various animal models of endotoxemia (Nishida et al., 1994; Huang et al., 1997). The use of selective iNOS inhibition produced equivocal results. In endotoxemic pigs, the selective iNOS inhibi-
tor aminoethyl isothiourea normalized hepatic artery blood flow and partially restored portal venous flow (Saetre et al., 1998). The same inhibitor, however, caused a dramatic decrease in liver microvascular blood flow in a rat ischemia–reperfusion/endotoxemia model (Wang et al., 1998). In contrast, evidence from NO donor studies in experimental sepsis suggest that low to moderate levels of NO may enhance global liver perfusion and protect the hepatic sinusoid (Gundersen et al., 1998; Pastor et al., 1995b; Wright et al., 1992). A relative scarcity of endothelial NO due to endotoxin- and mediator-induced EC dysfunction is likely to contribute to the early (micro) circulatory disorders of sepsis (Bauer et al., 1997).

Endothelin-1 (ET-1) is a 21-amino-acid polypeptide produced by ECs. Increased ET-1 levels have been demonstrated in animal (Lundberg et al., 1991; Miura et al., 1996) and human (Lundberg et al., 1991) sepsis. This potent vasoconstrictor is thought to compensate for the systemic vasodilatation in septic shock and can induce endothelial NO release (Higuchi and Satoh, 1997). However, ET-1 can cause intense and sustained splanchnic vasoconstriction and has been found to impair sinusoidal blood flow in endotoxin-primed animals Bauer et al., 1994). This vasoconstrictive effect at the liver sinusoid level is mediated by the constriction of perisinusoidal stellate cells that exchanged their storage function for an activated contractile state (Sakamoto et al., 1993). It is suggested that sepsis sensitizes the hepatic microcirculation to ET-1 and that subsequent local imbalances between inducible NO and ET-1 further mediate altered microvascular responses (Baveja et al., 2002). However, in porcine septic shock, the endothelin receptor bosentan significantly improved microcirculatory blood flow in all splanchnic organs except the liver (Krejci et al., 2003), suggesting that ET-1 blockade did not effectively dilate the hepatic microcirculation.

Another molecule of interest in regulating the hepatic microcirculation is carbon monoxide (CO). CO is generated by heme oxygenase (HO) and acts by means of activation of guanylate cyclase. It dilates the sinusoid by means of relaxation of stellate cells (Suematsu et al., 1995). In a murine model of early ischemia–reperfusion induced systemic inflammation, exposure to exogenous CO protected the hepatic microvasculature and improved impaired liver cell integrity and hepatocellular redox state (Wunder et al., 2005). Inhibition of HO ameliorated sepsis-induced liver dysfunction in rats.
that only vaguely reflect microcirculatory alterations. 

Microvascular perfusion in vivo has been extensively studied in animals. However, extrapolating these data to the clinical arena is hampered by the lack of adequate visualization techniques. The clinician must rely on indirect or “downstream” parameters such as lactate levels, assessment of intestinal–arterial pH and PCO2 gradients, or mixed venous oxygen saturation measurement that only vaguely reflect microcirculatory alterations. Recently, orthogonal polarization spectral (OPS) imaging (CytoscanTM, Cytometrics, Philadelphia) has been introduced and validated for clinical application (Mathura et al., 2001). The OPS technique is incorporated in a small hand-held device that can be used at the bedside. It enables the evaluation of microvascular blood flow in tissues covered by a thin epithelial layer. Of these, the sublingual area has been most extensively studied. Based on this technique, several studies in severe sepsis and septic shock have sustained the concept that microvascular alterations are associated with organ failure and, if persistent, are indicative of poor outcome (De Backer et al., 2002; Sakr et al., 2004). One major drawback is that the sublingual microcirculation is assumed (Fries et al., 2006) but not proven (Boerma et al., 2007) to behave like the hepatosplanchnic microcirculation.

**EFFECT OF TREATMENT ON THE (HEPATO)SPLANCHNIC MICROVASCULAR DURING SEPSIS AND SEPTIC SHOCK**

Restoring normal microvascular flow and function represents a crucial challenge in the prevention and treatment of sepsis-induced multiorgan failure. The impact of a combined goal-directed treatment strategy for sepsis on the microvascular environment remains speculative and may even be inadequate. The detrimental effects of nonselective NOS inhibition on the microcirculation shown in the experimental setting are corroborated by a negative phase III placebo-controlled clinical trial using a nonspecific NOS inhibitor L-NMNA in septic shock patients. The trial was terminated prematurely due to a higher incidence of cardiovascular failure and an increased mortality in the treatment arm (Lopez et al., 2004). NO donors and selective iNOS inhibitors have not been used in human sepsis.

Therapies that target the endothelium and specifically combat the inflammatory and coagulation cascade might reduce microcirculatory failure. In this context, corticosteroids have been put forward. Stress doses of hydrocortisone favorably modulate host defense and reduce bacterial colonization of the liver during endotoxemia (Heller et al., 2003). Simultaneous administration of hydrocortisone and inhaled NO attenuated the inflammatory response and almost restored normal liver morphology in porcine endotoxic sepsis (Da et al., 2007). However, outcome studies of steroid treatment in clinical sepsis and septic shock are inconclusive (Annane et al., 2004; Britt et al., 2006). Pre- and posttreatment with antioxidant drugs protected against liver damage, attenuated endothelial dysfunction, and improved sinusoidal perfusion in endotoxemic animals (Schmidt et al. 1997; Spapen et al., 1999; Hsu et al., 2006), but none of these effects was ever reproduced in clinical studies. An increasing body of evidence supports activated protein C (APC) as the “missing link” in the approach of sepsis-induced microvascular failure. Through binding on specific receptors, APC closely interacts with ECs and circulating macrophages. APC is thought to attenuate the inflammatory response in ECs and monocytes (Joyce and Grinnell, 2002) and inhibits the adherence of leukocytes to ECs (Iba et al., 2005). APC also has anti-apoptotic activity (Bilbault et al., 2007) and enhances microvascular perfusion by its inherent antithrombotic and profibrinolytic actions (Dhainaut et al., 2003). APC increased intestinal microvascular perfusion in experimental endotoxemia (Lehmann et al., 2006). In severe clinical sepsis, the administration of APC rapidly and consistently improved sublingual microvascular alterations, whereas its cessation caused transient deterioration (De Backer et al., 2006). These findings were independent of changes in systemic variables, including arterial pressure and cardiac output. APC actions on the hepatic microvasculature have not been documented. A large phase III multicenter placebo-controlled trial in severe clinical sepsis found a 6% absolute decrease in mortality in the APC-treated patients (Bernard et al., 2001). APC has been commercialized as Xigris™ (Eli Lilly Company) for the treatment of severe sepsis with organ failure.

**LITERATURE CITED**


