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Role of endothelin ET_A receptors in sepsis-induced mortality, vascular leakage, and tissue injury in rats

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Abstract

The role of endothelin ET_A receptors in sepsis-induced mortality and edema formation was evaluated with a selective antagonist ABT-627 [2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-di(*n*-butyl)amino carbonylmethyl)-pyrrolidine-3-carboxylic acid]. Sprague–Dawley rats received saline (control group), *Escherichia coli* endotoxin (10 mg/kg, sepsis group) or infusion of ABT-627 prior and immediately after saline and endotoxin injection. Mortality, edema formation (wet/dry ratios), and multiple tissue injury (indicated by serum concentrations of creatinine, urea, bilirubin, creatine kinase, lactate dehydrogenase, and aspartate aminotransferase) were monitored within 5 h. Endotoxin injection elicited 64% mortality, significantly augmented edema formation in liver, heart, lung, and kidney, and raised serum levels of tissue injury markers. Pretreatment with ABT-627 completely reversed endotoxin-induced mortality, significantly attenuated wet/dry ratios of the heart, liver, and kidney, but not lungs, and reduced serum levels of creatine kinase, creatinine, aspartate aminotransferase, and lactate dehydrogenase, but not that of urea and bilirubin. These results suggest that endothelin ET_A receptors play a significant role in promoting mortality, edema formation (except in the lungs), and tissue injury in animals with severe sepsis.

Keywords: Sepsis; Septic shock; Endothelin; Endothelin receptor; Cardiac output; Organ failure; Edema

1. Introduction

Septic shock, a major cause of high mortality rate in intensive care units, is usually associated with multiple organ failure, peripheral vascular dysfunctions, maldistribution of blood flow among various organs, disseminated intravascular coagulation, leakage of plasma into the interstitial space, and endothelial cell injury. Bacterial lipopolysaccharides, the outer membrane of Gram-negative bacteria, is widely accepted as a central player in the pathogenesis of septic shock by activating the release of mediators and cytokines from various cells. Several mechanisms have been implicated in pathogenesis of vascular dysfunctions and organ injury associated with septic shock, including direct inhibition of the sympathetic system by circulating endotoxin, direct suppression of vascular smooth muscle metabolism, increased local metabolic demands and the release of pro-inflammatory cytokines (Bone, 1991), and lately, enhanced local release of vasoactive mediators such as prostaglandins, thromboxanes, nitric oxide, and endothelins (Battistini et al., 1996).

Endothelins are a family of acidic 21-amino acid peptides found in at least three distinct isoforms, endothelin-1, -2, and -3, which share sequence homology and arise through proteolytic processing of pro-hormones (prepro-endothelins) (Inoue et al., 1989). Prepro-endothelins are proteolytically cleaved to form pro-endothelins (big endothelins) which are between 38 and 41 amino acid peptides long (Inoue et al., 1989). These, in turn, are cleaved by two isoforms of enzymes known as endothelin-converting enzymes to form mature endothelin peptides. Endothelins exert numerous biological actions by acting through two main G-protein coupled receptor families, endothelin ET_A and ET_B receptors. Both of these receptors stimulate phospholipase C which leads to increased formation of diacylglycerol and inositol-1,4,5-trisphosphate which, in turn,

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activates protein kinase C pathway and increases intracellular Ca^{2+}, respectively (Simonson and Dunn, 1990).

Many investigators have reported that circulating endothelin levels increase significantly in septic humans and animals and that these levels correlate with mortality (Weitzberg et al., 1991; Pittet et al., 1991). Both increased local production of endothelins and poor pulmonary and renal clearance of endothelins have been blamed for the rise in circulating endothelins in septic shock. Recent evidence suggests that endothelins play a significant role in vascular dysfunctions and organ failure associated with sepsis and septic shock. Indeed, several reports have illustrated that antagonism of endothelin receptors in septic animals led to amelioration of metabolic acidosis and improvement of coronary, renal, splanchnic, pulmonary, and intestinal perfusion (Mitaka et al., 1998; Wanecek et al., 1997a, 2001; Oldner et al., 1999; Albertini et al., 2001). However, whether endothelin ET_A receptors or ET_B receptors are largely responsible for the deleterious role of endothelins in sepsis remains unclear. This is because investigators have so far used mainly nonselective endothelin ET_A/ET_B receptor antagonists such as bosentan [4-*tert*-butyl-*N*-(6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2,2′-bipyrimidin-4-yl)-benzenesulphonamide], SB209670 [(+)-1*S*,2*R*,*S*-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylendioxyphen-yl)-5-prop-1-yloxyindane-2-carboxylate], and TAK-044 (cyclo[D-α-aspartyl-3-[(4-phenylpiperazin-yl)carbonyl]-L-alanyl-L-α-aspartyl-D-2-(2-thienyl) glycyl-L-leucyl-D-tryptophyl] disodium salt). In addition, even when selective endothelin ET_A or ET_B receptor antagonists were used, the influence of these interventions on the function of only one organ was evaluated in septic animals (Schmeck et al., 2000; Wanecek et al., 1999; Oldner et al., 1999).

One possible mechanism through which endothelins participate in the pathogenesis of septic shock and severe sepsis is enhanced microvascular permeability and reduction of circulating blood flow. Both abnormalities are known to contribute to multiple organ failure and high fatality in humans or animals with septic shock. Exogenous endothelin-1 induces albumin extravasations in various organs and reduces plasma volume (Filep et al., 1991), effects which are mediated through endothelin ET_A receptor activation (Filep et al., 1994). However, the involvement of endothelins in sepsis-induced increased microvascular permeability and edema formation in various organs remains unclear primarily because only few investigators have measured the effect of endothelin ET_A receptor antagonists on edema formation in various organs, and only the immediate effect of endotoxin or sepsis on vascular injury has been addressed (Filep, 2000).

In this study, we evaluated the selective role of endothelin ET_A receptors in mediating mortality, multiple tissue injury, and edema formation in various organs elicited by lipopolysaccharides injection in rats. Our objective of selective antagonism of endothelin ET_A receptors was achieved by ABT627 [2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(*N*,*N*-di(*n*-butyl)amino carbonylmethyl)-pyrrolidine-3-carboxylic acid], a highly selective, potent nonpeptidic endothelin ET_A receptor antagonist (Winn et al., 1996). The pharmacokinetics and the selectivity of this antagonist to block endothelin ET_A receptors selectively both in ex vivo and in vivo conditions have already been described (Wessale et al., 2002). Our results clearly indicate that administration of ABT-627 completely prevented sepsis-induced mortality and significantly attenuated edema formation and serum indicators of multiple tissue injury.

2. Methods

2.1. Animal preparation

The Animal Research Committee of McGill University approved all procedures. Adult pathogen-free male Sprague–Dawley rats (450–550 g) were used. The animals were housed in the animal facility of the hospital, were fed food and water ad libitum, and were studied 1 week after arrival. All animals were lightly anesthetized with sodium pentorbarbital (30 mg/kg) and were given supplemental doses (10 mg/kg) as needed (based on the absence or presence of corneal reflexes). The animals were tracheostomized with polyethylene tubing (internal diameter of 2.2 mm) which was sutured firmly in place with a silk tie. Tracheal pressure was measured with a differential transducer (Validyne, Northridge, CA), which was connected to the tracheal catheter via a side-port. A catheter (22 gauge), placed into the jugular vein, was used to access the venous circulation.

2.2. Survival experiments

At the end of the surgical procedure, a 1-h stabilization period was allowed. The animals were then divided into four groups. Group 1 ($n=8$) served as control and received an intraperitoneal (i.p.) injection of normal saline, whereas group 2 ($n=6$) received ABT-627 (a selective endothelin ET_A receptor antagonist) (generously provided by Abbott Laboratories). ABT627 was dissolved in normal saline and was injected intravenously as a single bolus (1 mg/kg) followed by constant infusion (0.01 mg/kg/min) for 30 min prior to saline injection and for an additional 30 min after saline injection. Group 3 ($n=8$) received intravenous injection of *Escherichia coli* lipopolysaccharides (serotype 055:B5, Sigma, 10 mg/kg). The fourth group of animals ($n=12$) received ABT-627 as a single bolus (1 mg/kg) followed by constant infusion (0.01 mg/kg/min) for 30 min prior to lipopolysaccharides injection and for an additional 30 min after lipopolysaccharides injection. Volume of infusate was kept to a minimum (not exceeding 100 μl). Our preliminary experiments revealed that at this dosing regiment, ABT627 was capable of blocking the pressor response elicited by a bolus injection of endothelin-1 (0.3 nmol/kg) administered at 1 and 4 h post-dosing. Survival of

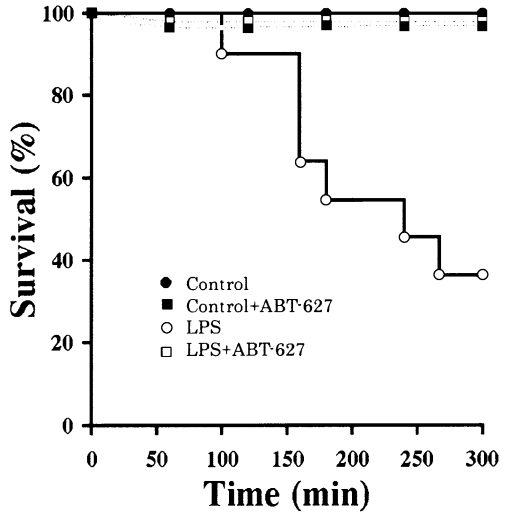

Fig. 1. Changes in animal survival in the four groups of animals. LPS: lipopolysaccharides. Note that lipopolysaccharides injection elicited a progressive decline in animal survival through the 5-h experimental period, whereas administration of ABT-627 along with lipopolysaccharides completely abrogated lipopolysaccharides-induced mortality. ABT-627 infusion in control animals had no effect on survival.

animals was monitored for a period of 5 h, and death was identified by cessation of respiratory activities (monitored with tracheal pressure).

2.3. Edema formation and tissue injury experiments

Four groups of animals were studied as described above. Group 1 ($n=6$) received normal saline and served as control, whereas group 2 ($n=6$) received ABT-627 prior to saline injection in a dosing regimen identical to that described in the survival experiment. Group 3 ($n=14$) animals were injected intravenously with *E. coli* lipopolysaccharides as described above. Larger numbers of animals were studied in this group because of the high mortality elicited by lipopolysaccharides injection (see Results). The involvement of endothelin ET_A receptors in lipopolysaccharides-induced vascular leakage and tissue injury was assessed in group 4 ($n=6$) animals in which ABT-627 was administered prior to lipopolysaccharides injection as described in the survival experiment. At the end of the 5-h experimental period, 1.5 ml of blood was collected from the venous catheter and was centrifuged (6000 rpm for 5 min) to separate serum. All serum samples were analysed in the Clinical Biochemistry Laboratory of Royal Victoria Hospital, McGill University. The following marker enzymes were measured in the serum as biochemical indicators of multiple organ dysfunction syndrome: (1) Liver dysfunction and failure were assessed by measuring the rise in serum aspartate aminotransferase (a nonspecific marker for hepatic injury) and bilirubin (an indicator of hepatic excretory function and predictor of the development of liver failure); (2) Renal dysfunction and failure was assessed by evaluating the rise in serum creatinine (an index of reduced glomerular filtration rates and renal failure) and urea (an indicatory of increased catabolism and/or reduced excretory function of the kidney); (3) Cardiac and skeletal muscle injury was assessed by measuring total creatine kinase level; (4) Lactate dehydrogenase concentration was measured as a general index of tissue injury. After removal of blood samples, the vasculature was flushed with phosphate buffer

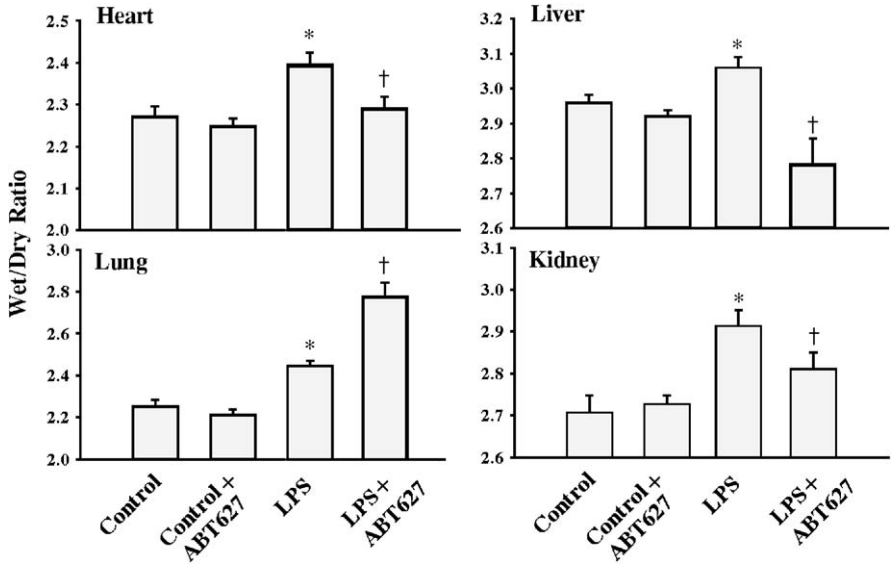

Fig. 2. Changes in wet/dry ratio of various organs in the four groups of animals. $^{*}P<0.05$ compared with the control group. $^{+}P<0.05$ compared with the lipopolysaccharides group. Note that administration of ABT-627 reversed lipopolysaccharides-induced rise in heart, liver, and kidney wet/dry ratios but had the opposite effect on lung wet/dry ratio.

solution injected into the venous catheter. Liver, kidney, heart, and lungs were then excised, weighed (wet weight), and then dried in an oven at 50 °C for 12 h followed by measurement of dry weight.

3. Results

Fig. 1 shows animal mortality observed within the 5-h experimental period in the four groups of animals. Injection of lipopolysaccharides elicited a significant and a progressive decline in survival, with only 36% of animals surviving after 5 h of lipopolysaccharides injection. Infusion of ABT-627 along with lipopolysaccharides completely reversed lipopolysaccharides-induced mortality, and none of the animals in this group died during the experimental period (Fig. 1). No mortality was observed among control animals and control animals infused with ABT-627 (Fig. 1).

Fig. 2 illustrates the influence of lipopolysaccharides injection in the presence and absence of endothelin ET_A receptor blockade on wet/dry ratios of various organs. Lipopolysaccharides injection elicited a significant increase in wet/dry ratio of the heart, liver, kidney, and lungs ($P<0.05$ compared with control values, Fig. 2). Infusion of ABT-627 along with lipopolysaccharides resulted in a significant decline in wet/dry ratio of the heart, kidney, and liver compared with that measured with lipopolysaccharides alone ($P<0.05$); however, lung wet/dry ratio actually rose significantly higher than that measured with lipopolysaccharides alone ($P<0.05$, Fig. 2). Infusion of ABT-627 in control animals had no significant effects on wet/dry ratios of the heart, liver, kidney, and lungs (Fig. 2).

Serum analysis of the surviving animals over the 5-h experimental period ($n=6$ in the lipopolysaccharides group) revealed that lipopolysaccharides injection elicited a substantial rise in serum aspartate aminotransferase, total creatine kinase, lactate dehydrogenase, creatinine, total bilirubin, and urea (Fig. 3). Antagonism of endothelin ET_A receptors with ABT-627 significantly attenuated the rise in serum aspartate aminotransferase, total creatine kinase, lactate dehydrogenase, and creatinine ($P<0.05$

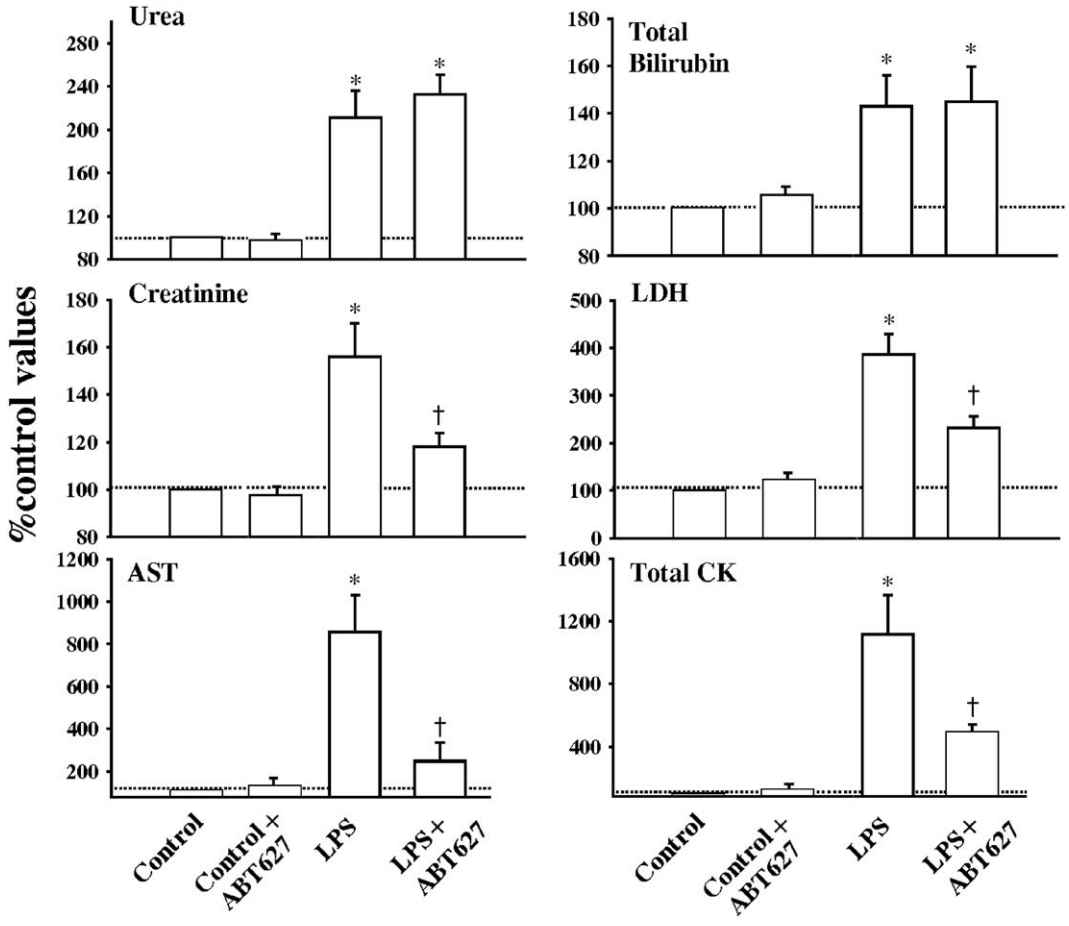

Fig. 3. Changes in serum levels of various indicators of tissue injury in the four groups of animals. Values are expressed as % of control animals. Symbols are as in Fig. 2. Note that ABT-627 had no effect on serum indices of tissue injury in control animals. Infusion of ABT-627 in septic animals attenuated lipopolysaccharides-induced elevation of lactate dehydrogenase, creatinine, aspartate aminotransferase, and total creatine kinase but had no effect on elevated levels of urea and total bilirubin.

compared with lipopolysaccharides alone) but had no effect on total bilirubin and urea concentrations (Fig. 3). Note that infusion of ABT-627 in control animals had no effects on any of the serum markers of tissue injury (Fig. 3).

4. Discussion

The main finding of this study was that infusion of a selective endothelin ET_A receptor antagonist (ABT-627) along with lipopolysaccharides completely reversed lipopolysaccharides-induced mortality, attenuated edema formation in the liver, heart, and kidney, and reduced the levels of serum markers of tissue injury and dysfunction (except for urea and bilirubin).

4.1. Endothelins and tissue injury

Investigators studied functional roles of endothelins in septic shock by using nonselective endothelin ET_A/ET_B receptor antagonists such as bosentan, SB209670, and TAK044 and provided conflicting results. Bosentan injection in septic pigs improved survival, cardiac index, restored arterial pressure, enhanced systemic oxygen delivery, and acid–base status and completely abolished lipopolysaccharides-induced pulmonary hypertension and alterations in lung mechanics (Albertini et al., 2001; Wanecek et al., 1997a,b, 2001). Similar results were obtained with TAK044 (Ishimaru et al., 2001; Mitaka et al., 1998). By comparison, pretreatment of septic rats with SB209670 (nonselective ET receptor antagonist) resulted in worsening of mortality, hypotension, hepatocellular injury, and no improvement in kidney function (Ruetten et al., 1996; Gardiner et al., 1995). Treatment with BQ788 (*N-cis*-2, 6-dimethyl-piperidinocarbonyl-L-gamma-MeLeu–D-Trp (COOCH(3))-Nle) (a selective endothelin ET_B receptor antagonist) attenuated arterial hypotension, improved vascular reactivity to norepinephrine, and prevented liver injury (Ruetten and Thiemermann, 1996). By comparison, another endothelin ET_B receptor antagonist (A-192621) (2-(4-propoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-(2,5-ethylphenyl)aminocarbonylmethyl)-pyrrolidine-3-carboxylic acid) significantly increased lipopolysaccharides-induced mortality in pigs (Oldner et al., 1999; Wanecek et al., 1999).

We report here for the first time that antagonism of endothelin ET_A receptors in rats completely prevents lipopolysaccharides-induced mortality and attenuates serum indices of myocardial, renal, liver, and lung injury. Previous studies evaluated the effects of endothelin ET_A receptor antagonists on injury and dysfunction of a single organ and confirmed that the net effect of endothelins in sepsis is severely cardiodepressive (Wanecek et al., 1997a, 2001). The reduction in serum creatine kinase level in our study in response to ABT-627 infusion strongly suggests that endothelin ET_A receptor activation promotes myocardial injury in septic animals. These results also indicate that the reported beneficial effect of nonselective endothelin ET_A/ET_B receptor antagonists such as bosentan on myocardial performance may be mediated primarily through blockade of endothelin ET_A receptors. We should also emphasize that attenuation of total serum creatine kinase levels in septic rats may reflect a decline in the degree of skeletal muscle injury which our previous study have indicated could also be a target of elevated endothelin levels in septic animals (Guo et al., 1999).

Endothelin-1 decreases glomerular filtration rate and the ultrafiltration coefficient in renal glomeruli (Badr et al., 1989). Elevation of serum creatinine (Fig. 3) coupled with the reported increase in circulating endothelin-1 levels in septic animals and humans suggest that endothelin-1 is involved in reducing glomerular filtration rate in sepsis. This proposal is strongly supported by our observation of a significant attenuation of serum creatinine by ABT-627 administration in septic animals. This reduction in serum creatinine by ABT-627 could be mediated through inhibition of the direct effect of endothelin ET_A receptors and/or increased endothelin ET_B receptor sensitivity (Woodcock and Land, 1992). It is interesting that blockade of endothelin ET_A receptors with ABT-627 had no effect on lipopolysaccharides-induced elevation of serum urea levels. Although this observation might be interpreted as that endothelin ET_A receptors have no role in impaired excretory function of the kidney, alternative interpretation points to enhanced cellular catabolism as a cause of the significant rise in serum urea of septic animals and that endothelin ET_A receptors are not a major contributor to increased cellular catabolism.

The role of endothelins in sepsis-induced liver dysfunction is not clear. Blockade of endothelin ET_B but not ET_A receptors attenuates hepatocellular injury in endotoxemic rats (Ruetten and Thiemermann, 1996; Nishida et al., 1998). By comparison, nonselective endothelin ET_A/ET_B receptor antagonists ameliorated sepsis-induced hepatic injury and portal hypertension (Gandhi et al., 2001). Our study clearly illustrates that ABT-627 reduced serum aspartate aminotransferase but not bilirubin levels in septic animals, suggesting that endothelin ET_A receptors mediate hepatic tissue injury but not hepatic excretory dysfunction. The reasons behind the differences between our results in terms of liver injury and previous studies using another selective endothelin ET_A receptor antagonist (Ruetten and Thiemermann, 1996) are not clear. Species differences, variations in hemodynamic profiles of various septic shock models, the in vivo effectiveness and selectivity of endothelin-receptor antagonists, and the timing of administration of these antagonists during the course of septic shock could be involved. Moreover, one should also take into consideration the degrees to which the production and expression of endothelins and their receptors are elevated in animal models of sepsis and septic shock.

4.2. Endothelins and edema formation

Our study confirms that microvascular permeability is enhanced in septic rats as indicated by the significant rise in wet/dry ratios of the lungs, heart, kidney, and liver (Fig. 2). The fact that ABT-627 administration attenuated the rise in liver, heart, and kidney wet/dry ratios of septic animals suggests a strong role for endothelin ET_A receptors in promoting permeability in these organs. Filep (2000) has recently described that bosentan or FR139317 (2(*R*)-[2(*R*)-[2(*S*)-[(1-hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1*H*-indolyl)propionyl]amino-3-(2-pyridyl)propionic acid) (endothelin ET_A receptor antagonist) attenuated the acute (within 1–2.5 h) reduction in plasma volume and the rise in organ albumin escape rates in septic rats except in the lung and kidney. Our results regarding edema formation in the heart, liver, and lung are in agreement with that of Filep (2000). The apparent effectiveness of ABT-627 in ameliorating the rise in kidney wet/dry ratio in septic rats and the lack of effect of FR139317 on kidney albumin escape in Filep's experiment could be attributed to differences in the duration of sepsis in the two experiments (5 h in our study vs. 2.5 h in Filep's study), and consequently, different degrees of microvascular permeability changes in the kidney might have occurred among the two studies. In addition to inhibition of the direct effect of endothelin-1 on microvascular permeability, reduction in tissue wet/dry ratio with ABT-627 in our study could have also been mediated through reduction in mean arterial pressure and lowering of microvascular hydrostatic pressure. The apparent immunity of edema formation and albumin escape from the effect of endothelin ET_A receptor antagonists suggests that endothelins do not play a major role or may even mediate a protective role in the regulation of pulmonary microvascular permeability.

In summary, our results indicate that infusion of a selective endothelin ET_A receptor antagonist (ABT-627) completely prevents lipopolysaccharides-induced mortality and significantly attenuates both edema formation in various organs and serum levels of various indicators of tissue injury.

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