1. **ABSTRACT**

Despite advances in the understanding of pathophysiological mechanisms, there are limited pharmacotherapeutic options for sepsis, septic shock, and related pathologies. It is surprising that although sepsis-induced myocardial depression is documented in clinics, the cellular mechanisms are from clear. Alterations in molecular signaling mechanisms activated by cytokines and potent mediators such as ET-1 could pose the risk for myocardial dysfunction in sepsis. Our laboratory data suggest that the septic heart, in vivo, exhibits an increased time constant of left ventricular relaxation, $\tau$, along with changes in LVEDP. We also observed that bigET-1-induced elevation of ET-1 correlates with cardiodynamic alterations, induction of apoptosis, and activation of p38-MAPK phosphorylation during sepsis. In light of these evidences, we emphasize that these molecular alterations in heart, both at organ and cellular level during early sepsis, need to be elucidated thoroughly.
Sepsis-induced myocardial dysfunction

Table 1. Pro-inflammatory and anti-inflammatory mediators in sepsis

<table>
<thead>
<tr>
<th>Pro-inflammatory mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>• TNF-alpha</td>
</tr>
<tr>
<td>• IL-1</td>
</tr>
<tr>
<td>• IL-6</td>
</tr>
<tr>
<td>• IL-8</td>
</tr>
<tr>
<td>• PAF</td>
</tr>
<tr>
<td>• Leukotrienes</td>
</tr>
<tr>
<td>• Thromboxane A2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-inflammatory mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>• IL-4</td>
</tr>
<tr>
<td>• IL-10</td>
</tr>
</tbody>
</table>

TNF, Tumor necrosis factor; IL, Interleukin; PAF, Plasminogen activating factor.

2. INTRODUCTION

2.1. Sepsis

Sepsis is a complex progressive immunological, metabolic, and cardiovascular disorder that results from dysregulation of normally protective anti-microbial host defense mechanisms, followed by the development of Systemic Inflammatory Response Syndrome (SIRS) in patients (1). Sepsis is a major cause of morbidity and mortality in intensive care units (ICUs), particularly in elderly, immunocompromised, and critically ill patients, worldwide (2, 3). The mortality from septic shock syndrome ranges between 20-90% depending upon the patient’s age and associated pathologies (4). The incidence of sepsis has increased during last 20 years, with more than 500,000-1 million patients developing sepsis each year in the US. According to the National Vital Statistics Report (2005), septicemia and sepsis are now the 10th leading cause of death as opposed to being the 13th leading cause of death in 1990 in the US (5). Sepsis can cause multiple organ dysfunction, including cardiovascular complications because of the vicious cycle of inflammation and coagulation and often leads to death.

2.2. Pathophysiology

Sepsis is triggered by localized tissue infection or direct introduction of microorganisms into the bloodstream (e.g., via intravenous catheters). The septic response may also be induced by microbial exotoxins that act as superantigens (for example, toxic shock syndrome toxin). In septic shock, bacterial infection causes circulatory insufficiency when bacterial products interact with host cells and serum proteins to initiate a series of reactions that ultimately may lead to cell injury and death. These bacterial products themselves are deleterious, and the widespread and upregulated host response to these substances results in the elaboration of an extensive array of chemical mediators that lead to further cell damage. Various systems and mediators are stimulated in septic shock, including arachidonic acid metabolites (e.g., leukotrienes, prostaglandins, thromboxanes), complement system, coagulation cascade, fibrinolytic system, catecholamines, glucocorticoids, prekallikrein, bradykinin, histamines, beta-endorphins, enkephalins, adrenocorticoid hormone, circulating myocardial depressant factor(s), cachectin (tumor necrosis factor), interleukin-1, endothelin-1 (ET-1) etc. (Table 1, Figure 1). In the current review, we will discuss the role of ET-1 mediated signaling mechanisms in myocardial dysfunction during sepsis and septic shock.

2.3. Animal models of sepsis

One of the foremost challenges that the scientific community has faced in sepsis research is the availability of a clinically relevant model of sepsis. Various models of sepsis are available that replicate some signs and laboratory findings observed clinically. These models can be generally classified as infection models, intravenous infusion models, and endotoxosis models (6). Although these models mimic several key indications as observed in clinical sepsis, the driving factor is the extent of replication of the clinical and physiological features of septic patients. Witcherman et al (7) have outlined the following guidelines for experimental septic models:

1. The animals should show clinical signs of sepsis such as malaise, fever, chills, generalized weakness, etc.
2. The septic insult should occur over a period to allow the animal to respond to the insult and attempt to overcome the challenge.
3. The model should be reproducible to allow a majority of the prepared animals to be available for the study.

2.3.1. Intravenous infusion of live bacteria and endotoxosis model

In these models, bolus or short-term continuous infusion of large doses of bacteria (10^9 - 10^10/kg) are administered intravenously (8). The bolus intravenous infusion of live microorganisms does not simulate clinical sepsis. In clinical sepsis, the release of endotoxin into the bloodstream is intermittent while the infusion of microorganisms produces an abrupt immune challenge. This model produces a rapid decrease in cardiovascular function and cardiac output with severe mortality within hours (8). There is a transient increase in serum cytokine concentrations and the magnitude is greater than that observed clinically (9). The antisepsis agents that were not clinically effective showed a positive outcome in this model. Thus, this model does not provide clinically relevant information, even when primates are used as the experimental species.

Endotoxosis models are also widely used as they are easier to perform, and endotoxin is an important agent that induces sepsis. Models that use sublethal doses of endotoxin induce a hyperdynamic response. The continuous infusion model of endotoxin is also an advantageous model because it produces a persistent physiologic response. However, the route of administration of endotoxin is a key factor in this model. For instance, intraperitoneal endotoxin induces a hyperdynamic response in response to endotoxin. Thus, to ensure that the endotoxosis model is clinically relevant, the physiologic variables such as mortality rate, etc. must be clearly defined (6).

2.3.2. Peritonitis model

The peritonitis models that include cecal ligation and puncture (CLP), cecal inoculum, and fecal peritonitis models have been used in both small and large animals.
Figure 1. Pathogenesis of organ dysfunction during sepsis.

The peritonitis models induce an initial hyperdynamic cardiovascular response. The animals exhibit indications of systemic sepsis that progressively deteriorates, causing cardiovascular dysfunction and death several days after the initial insult. The serum cytokine concentrations in animals made septic using peritonitis models are similar to that observed clinically (9).

2.3.3. Fecal peritonitis

In fecal peritonitis model, bacteria suspended in a fibrin clot are implanted into the peritoneal cavity of the dog (10). However, the infectious insult in this model appears artificial and the failure to surgically treat the focus of infection in this model makes it resemble to incompletely treated peritonitis. In a study performed in male Yucatan minipigs, Kazarian et al (11) compared the effect of autologous fecal inoculum (FEC) and a pure culture of Escherichia coli (EC). The authors concluded from the study that i.p. EC models evoke a systemic response similar to i.v. administration of LPS or EC; however, the FEC model produced a systemic response akin to a slower developing septic process.

2.3.4. Cecal ligation and puncture (CLP) model

CLP is one of the most widely accepted and used animal model of sepsis. It satisfies the requirement of an episodic release of microorganisms into the blood stream as observed clinically. However, the model produces a rapidly lethal septic state, with mortality varying from 100% at 16-24h to 77% at five days depending on the number of punctures and size of needle used to induce sepsis. Although used widely because of clinical relevance, the CLP model also presents some disadvantages, include variation in mortality as it appears to be a personnel-dependent model.

2.3.5. Cecal inoculum model

To limit inter-personal variability and mortality in different laboratory conditions of the CLP model, cecal inoculum model was developed. The method involves an intraperitoneal administration of a quantified dose of cecal contents (40 mg/ml) obtained from a donor rat at a concentration of 200 mg/kg (12-23) (Figure 2). The cecal dose can be increased to accommodate fluid resuscitation, which greatly expands the versatility of this model (24, 25). The animals made septic via the cecal inoculum method exhibit piloerection, lethargy, periocular discharge, epistaxis, lack of grooming, lose stools, etc. Evaluation of the peritoneal cavity reveals the presence of ascites and infarcts on the peritoneal organs. The animals also show leukopenia and lactic acidosis at 24 and 48-h post-sepsis induction (14). In addition, induction of sepsis by this method produces an altered profile of steroid hormones,
Sepsis-induced myocardial dysfunction

Figure 2. Induction of chronic peritoneal sepsis using cecal inoculum. Healthy donor rat is euthanitized to isolate cecal material. Cecal material is suspended in sterile 5% dextrose water (D5W) at a concentration of 200 mg/5 mL of D5W to yield cecal inoculum (C.I.). Sepsis is induced in rats using intraperitoneal (i.p.) injection of C.I. using a 18 gauge needle. Animals receive 200 mg/kg of cecal inoculum i.p. After injecting the C.I., animals are gently massaged in the abdomen to evenly distribute the injectate. Sham animals receive an injection of sterile D5W at 5 mL/kg concentration.

particularly testosterone similar to that observed in septic patients (12, 18). The mortality observed in this model generally occurs before 24 h (5-15%) and between 5- and 7-days (40-60%). These septic animals also undergo a significant weight loss over a period of 48-72 h post-sepsis induction and do not regain baseline weight by day 7 (14).

At 24-h post-sepsis induction, the animals are hemodynamically stable but display tachycardia. By 3-days of sepsis induction, mean arterial pressure (MAP) is similar to the pre-sepsis values, but pulse pressure remains widened. This increase in pulse pressure is attributed to a significant reduction in diastolic arterial blood pressure (13). At 7-days following sepsis induction, there is a significant reduction in MAP and pulse pressure along with elevated HR, blood lactate, and WBC count. The physiologic parameters associated with this model are described in a review (14).

3. SEPSIS-INDUCED MYOCARDIAL DYSFUNCTION

Sepsis is associated with multiple organ dysfunction, including renal dysfunction, cardiovascular dysfunction, etc. (13, 26). Myocardial dysfunction manifests in septic patients in two phases. Phase 1: Hyperdynamic stage or warm shock, and Phase 2: Hypodynamic stage or cold shock (27-29). Patients in a hypodynamic stage exhibit increased vascular tone and low cardiac output (CO) along with cold clammy skin and a thready pulse (30, 31). A hyperdynamic stage of sepsis results after adequate fluid resuscitation. Patients in this stage show peripheral vasodilatation with a high cardiac output (30) and warm dry skin with a bounding pulse. Adult septic patients generally exhibit hyperdynamic shock while the pediatric septic population may present with either hypodynamic or hyperdynamic stage of septic shock (32).

3.1. Clinical manifestations

Various studies have shown that adequately resuscitated septic patients manifest a hyperdynamic circulatory state with high CO and low systemic vascular resistance (SVR) (31). This hyperdynamic state persists until death in non-survivor septic patients. Despite strong evidence depicting a compensatory hyperdynamic phase, various studies still suggest that the septic myocardium is dysfunctional, as illustrated by decreased left ventricular stroke work index (33). The depression in the Frank-Starling curve observed in these studies can be explained by either a change in myocardial contractility or compliance (31).

Calvin et al (34), for the first time demonstrated myocardial dysfunction in appropriately resuscitated septic patients using portable radionuclide cineangiography (RNCA). They showed increased left ventricular end-diastolic volume index (LVEDVI) and depressed left ventricular ejection fraction (LVEF) in a subgroup of five septic patients. Similarly, Parker et al (35), using serial RNCA and simultaneous thermodilution CO studies on a group of 20 septic patients, demonstrated a depression in LVEF despite normal or elevated cardiac index (CI) and SVR. The non-survivors maintained normal LVEF and LVEDVI throughout the course of the illness until death. This study documented two important aspects of sepsis-
induced myocardial dysfunction. First, septic shock survivors were more likely to exhibit acute left ventricular dilation (increased LVEDVI) and decreased LVEF than non-survivors, who typically maintained normal cardiac volumes and LVEF. Second, acute changes in ventricular volumes and LVEF associated with sepsis were sustained up to 4 days, and then returned to normal in 7-10 days in survivors. This suggests that myocardial depression in human septic subjects is reversible. Doppler echocardiography revealed abnormal diastolic filling properties in left ventricles of septic patients (36, 37). Similarly, transesophageal echocardiography demonstrated isolated diastolic dysfunction and combined systolic and diastolic abnormalities (38).

In the systemic circulation, sepsis produces decreased vascular resistance and blood pressure. The left ventricle afterload is usually depressed that tends to maintain or increase CO during left ventricular contractile dysfunction (30). In contrast, right ventricular afterload is elevated due to an increase in pulmonary vascular resistance associated with lung injury and adult respiratory distress syndrome (30). Parker et al (39) showed a decrease in right ventricle ejection fraction (RVEF) independent of pulmonary vascular resistance and pulmonary artery pressure. They suggested that increased right ventricular afterload could not be the dominant cause of right ventricular depression in septic shock. The study also demonstrated a close temporal parallel between right and left ventricular dysfunction. However, the contribution of the right ventricle towards sepsis-induced impaired cardiac function is unknown.

### 3.1.1. Myocardial prognostic factors during sepsis

Three hemodynamic patterns of mortality in septic shock have been observed (40). Early mortality is due to either distributive shock (low SVR and refractory hypotension despite preserved CI) or cardiogenic shock (decreased CI). It appears that the non-survivors die due to cardiogenic shock are unable to dilate their left ventricle, as illustrated by reduced LVEDVI and CI. Patients that have increasing LVEDVI and preserved CI succumb to classic distributive shock.

The introduction of the pulmonary artery catheter (PAC) that can measure pulmonary artery wedge pressure allows for a better reflection of cardiovascular dysfunction during sepsis (41). The studies using the PAC demonstrated that adequately-volume resuscitated septic patients exhibited high CI and low SVR, including non-survivors. Hence, CI is no longer considered as a reliable predictor of mortality in sepsis (31). Presence of low SVR post-resuscitation indicated that peripheral vascular failure could be a major determinant of mortality during septic shock. Parker et al (40) reviewed septic patients on presentation and at 24-h to identify a prognostic criterion. They showed that, on presentation, only a heart rate (HR) < 106 bpm suggested a favorable outcome. At 24-h, HR<95 bpm, SVR index (SVRI)=1529 dynes-sec/cm\(^2\)/m\(^2\), a reduction in HR=18 bpm and CI=0.5 L/min/m\(^2\) predicted survival. In addition, non-survivors of septic shock show an attenuated inotropic response to a dobutamine stress test (42). Increased SVRI, venous oxygen saturation, ventricular dilation, and a reduction in diastolic blood pressure in response to dobutamine stress test predict survival in septic patients (31).

### 3.2. Characterization of sepsis-induced myocardial dysfunction: is tau the main predictor?

Several research laboratories have demonstrated that sepsis-induced myocardial dysfunction occurs at cellular level in isolated organs and clinically in humans (13, 22, 38, 43-46). Although alterations in left ventricular relaxation and contraction have been found in isolated heart preparations in various animal models of sepsis, this effect was not seen in vivo, primarily due to activation of compensatory changes occurring locally or centrally in peripheral vascular perfusion, venous return, pulmonary artery wedge pressure, and/or heart rate (47, 48). Myocardial dysfunction has been characterized in several animal models of sepsis.

In a canine model of CLP, systolic (reduced ejection fraction) and diastolic (reduced compliance) abnormalities of left ventricular function have been demonstrated (49). Piper et al (50) have demonstrated cardiac injury in isolated hearts during CLP. These septic rats exhibited 1) reduction in the rate of increase and decrease in left-ventricular pressure (+dP/dt\(_{max}\) and –dP/dt\(_{max}\), respectively); 2) shift in the left-ventricular Starling curves towards right and downward; and 3) reduction in the ability of the heart to develop pressure. In a study performed at 3.5 h after induction of CLP, Field et al (51) have shown myocardial dysfunction as reduced stroke volume and stroke work. In the same study, it has been shown that hearts isolated from CLP-induced sepsis displayed depressed peak rate of ventricular pressure development and elevated ventricular stiffness. In a canine model of E. coli endotoxin infusion, reduction in left ventricular filling pressure and aortic pressure along with increase in heart rate was observed (52). Abnormalities in left ventricular diastolic dysfunction have also been reported in a porcine model of endotoxemia (53). In an E. coli endotoxin (LPS)-induced endotoxemia model in guinea pigs, Zhong et al (54) reported depressed LV compliance 4-h after induction of endotoxemia. They reported that intravascular volume expansion selectively improved LV diastolic compliance of LPS hearts without affecting LV systolic function. In a similar study, Adams et al (55) demonstrated that the LV mechanical disadvantage of hearts treated with endotoxemia was not correlated with changes in beating frequency, active state duration, or tissue water content; neither was it surrounded by pyruvate nor by maximally effective increases in coronary flow, diastolic stretch, or extracellular Ca\(^2+\) concentration. In another study, Parker et al (39) demonstrated that LV end-diastolic pressure-volume relationships in hearts isolated from endotoxemia challenged guinea pigs were shifted upward and to the left of controls in the direction of decreased diastolic compliance. Raymond (56) reported that in a canine model of endotoxemia, hearts showed a progressive energy deficit, whereas animals surviving the experimental protocol maintained levels of ATP and creatine phosphate.
Sepsis-induced myocardial dysfunction

Figure 3. Rate of change of left ventricular contraction (+dP/dt, A) & rate of change of left ventricular relaxation (–dP/dt, B) in isolated heart preparation (N=6) and contractile properties (C-F) of left ventricular myocytes isolated from control and septic (at 24 and 48-h) rats. Graphs depicted are (C) representative cell shortening traces (D) Peak shortening (E) maximal velocity of shortening (+dL/dt) (F) maximal velocity of relengthening (-dL/dt). Data are represented as mean ± s.e.m., n= 50 per data group, * P ≤ 0.05 as compared to control.

Similar to CLP model, in cecal inoculum model, Sharma et al (13) demonstrated reduced rates of left ventricular contraction and relaxation in isolated heart preparation (Figure 3A & B). In isolated ARVM, depressed contractility (reduced peak shortening and maximal velocities of shortening and relengthening) was evident during sepsis (22) (Figure 3C-F). We observed that septic hearts were also more susceptible to a calcium-paradox mediated myocardial injury (21). In our laboratory, similar to other investigators, we did not find any change in the rates of left ventricular relaxation and contraction, in vivo, at 24h post-sepsis (57). However, we have demonstrated that peritoneal sepsis causes a significantly elevated left ventricular isovolumic relaxation rate constant, \( \tau \) (57). This increase in \( \tau \) was accompanied by an elevated left ventricular end diastolic pressure (LVEDP). However, elevation in LV isovolumic relaxation rate constant, \( \tau \), has been reported in response to myocardial ischemia and heart failure (58). The prolongation of \( \tau \) occurs as a result of impaired LV relaxation (59). Patients with heart failure show increased \( \tau \) values with a leftward shift in the LV pressure volume relationship (60). Similar to these observations in patients, in an isolated rat heart preparation from septic animals, Farias et al (61) reported a leftward shift in the LV pressure volume relationship showing diastolic dysfunction and altered LV diastolic compliance.
Sepsis-induced myocardial dysfunction

These evidences corroborate our earlier data suggesting that sepsis produced LV contractile dysfunction during early stages.

LVEDP is a parameter employed to study left ventricular filling (62-64). Sepsis-induced increased peripheral vasodilatation (13) may lead to decreased stroke volume and thus decreased cardiac output. Physiologically, both at organ and vascular levels, the body compensates for the decrease in stroke volume by increasing heart rate to maintain cardiac output, leading to a hyperdynamic stage of sepsis (20). Increased heart rate in turn increases venous return and thus increases the left ventricular filling pressure as evident by increased LVEDP (48). Therefore, alterations of LVEDP during sepsis in the present study indicate progressive deterioration in myocardial function and suggest early stages of CHF. Sepsis is associated with the presence of systemic inflammatory response syndrome (SIRS). Symptoms of SIRS, including tachypnea and tachycardia, tend to increase the myocardial oxygen consumption to meet with altered metabolic state during sepsis’ pathophysiology (65). Researchers have suggested that even though LV function is impaired, myocardial oxygen consumption is increased (66). Increase in LV wall stiffness causes a leftward shift in the LV pressure volume curve, causing increased LVEDP at a lower LV volume (67). LVEDP is also suggested as one of the factors responsible for increased myocardial wall stress and eventual increase in myocardial oxygen consumption (48). Results obtained in our laboratory provided evidence that sepsis and septic shock-induced alterations in tau, LVEDP and RPP could be the first indicators for progression of myocardial dysfunction similar to that were seen during early stages of CHF.

4. MOLECULAR MECHANISMS

4.1. Cytokines

Induction of the innate immune system during systemic infection leads to an overwhelming production of proinflammatory cytokines. Both LPS and peptidoglycans from gram-negative and gram-positive bacteria interact with TLRs to activate transcription of cytokines and proinflammatory mediators like TNF-alpha, IL-6, etc. Although cytokines are not directly involved in controlling vascular responses, they are known to induce the production of vasoactive mediators such as nitric oxide and ET-1.

4.1.1. Tumor necrosis factor (TNF)

TNF, a proinflammatory cytokine, is a crucial early mediator of endotoxin-induced shock (13). TNF exists as two isoforms (TNF-alpha and TNF-beta) that share similar inflammatory activities (68). TNF-alpha is predominantly derived by activated macrophages, while TNF-beta is less potent and less abundant isoform that is produced primarily by T cells (69). Recent studies have shown that cardiac myocytes also secrete TNF-alpha in response to sepsis (70). An increased concentration of TNF-alpha has been shown to correlate with deficits in cardiac contraction and relaxation (71). In sepsis, the release of TNF-alpha in turn activates a second level of inflammatory cascades, including cytokines such as IL-6 and IL-8, lipid mediators, and reactive oxygen species, as well as up-regulating cell adhesion molecules (ICAM) that result in the initiation of inflammatory cell migration into tissues (72). TNF-alpha is detected within 20 minutes after an immune challenge (73). The concentration of TNF-alpha peaks between 90 minutes and 2 hours after endotoxin injection with transient release that varies in a wide range of concentrations (from few to thousands pg/ml) (74). TNF-alpha exists as a 17-kDa molecule and has a half-life of ~30 minutes and can be measured in both plasma and serum using either immunoreactive or cytotoxic assays (64). TNF-alpha is degraded in the liver, gastro-intestinal tract (GIT), and kidneys (73). TNF-alpha acts via two receptors designated as TNFR1 (p55) and TNFR2 (p75) (75). The concentration of soluble receptors for TNF-alpha is elevated during sepsis, particularly in patients with end-organ failure (76). However, studies using monoclonal antibodies directed against TNF-alpha or soluble TNF-alpha receptors failed to improve survival in septic patients (77-79).

4.1.2. Interleukins (ILs)

Interleukin-1 (IL-1) plays a central role in the systemic immune response and is implicated during sepsis and septic shock. IL-1, a 26-kDa protein is synthesized by monocytes, macrophages, and neutrophils in response to TNF-alpha. IL-1 has been shown to exert a negative inotropic effect and depresses cardiac contractility by stimulating nitric oxide synthase (80). Gene transcription of IL-1 is accompanied by a temporal delayed transcription of IL-1 receptor antagonist (IL-ra) that functions as an endogenous inhibitor of IL-1. Recombinant IL-ra has been evaluated in three multicentre trials that yielded a reduction in mortality by 4.9% in septic patients (81, 82). However, the clinical development of this protein as a therapy has been curtailed.

Another proinflammatory cytokine, interleukin-6 (IL-6) has been implicated in the pathogenesis of sepsis. IL-6 is produced by a variety of cells, including monocytes/macrophages, endothelial cells, fibroblasts, and smooth muscle cells in response to stimulation by LPS, TNF-alpha, and IL-1. IL-6 is considered a more consistent predictor of sepsis due to its prolonged elevation in the circulation than TNF-alpha (83).

4.2. Endothelin (ET)

4.2.1. ET-1 biology

ET mechanisms have been shown to play a very important role in the pathogenesis of sepsis (84). It has been reported that among all the pathophysiological conditions possibly involving the endothelial system, sepsis is associated with the highest levels of plasma ET-1 (85). Endotoxins increase plasma ET-1 levels, along with increased mRNA expression of preproET-1 in the lungs and heart (86, 87). Experiments involving infusion of ET-1 show signs of cardiovascular complications generally associated with septic shock (88, 89), thus suggesting that ET mechanisms may be the major contributive factor to the dysfunction of vital organs during sepsis (90, 91). These major findings suggest the importance of ET mechanisms
Sepsis-induced myocardial dysfunction

in sepsis. However, the role of ET-1 in the pathogenesis of sepsis-induced myocardial dysfunction is not yet defined.

The discovery of a peptide factor EDCF (endothelium-derived contracting factor) that exhibited vasoconstrictive property (92) stirred a major interest in the scientific community worldwide in 1985. This factor was characterized in a conditioned medium of cultured bovine endothelial cells. Yanagisawa et al (93) isolated, purified, sequenced, and cloned this EDCF from the supernatant of cultured porcine aortic endothelial cells and named it ET. This peptide is one of the most potent vasoconstrictors with a long-lasting action. It consists of 21 amino acids with a molecular weight of 2492 Daltons. This peptide with a free amino and carboxy terminal has four cysteine residues that form two intramolecular disulfide bonds (Cys1-Cys5; Cys3-Cys11).

The ET family consists of three isopeptides: endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). All three isopeptides are encoded by separate genes (94) but are localized differently. In humans, ET-1, ET-2, and ET-3 have been mapped to chromosome 6 [6p24-p23], chromosome 1 [1p34], and chromosome 20 [20q13.2-20q13.3] (95, 96) respectively. All the isoforms have vasoconstrictor action but at varied potency. ET-2 has vasoconstriction action similar to ET-1. ET-3, however, has reduced vasoconstrictor property in comparison to the other two isoforms (94). ETs have structural similarity to the sarafotoxins, which are cardiotoxic peptides isolated from the venom of Atractaspis engaddensis (94).

ET is produced by a multifarious group of cells and tissues. However, the expression of individual isoforms is tissue specific. Endothelial cells predominantly generate ET-1. ET-1 is also produced by cardiac myocytes, kidney, central nervous system, and human aortic smooth muscle cells (94). ET-2 is primarily produced within the kidney and intestine (97). Myocardium, placenta, uterus, and endothelial cells also generate ET-2 but at a lower concentration (97). ET-3 is predominately produced by the brain (98). The human ET-1 gene is composed of five exons, four introns, and 5’ and 3’-flanking regions distributed over 6,836 bp. Each of the five exons encodes a portion of preproET-1: exon 1 encodes the 5’ untranslated region and first 22 amino acids of the precursor; exon 2 encodes ET-1, first four residues of C-terminal of bigET-1 and the Trp-Val cleavage site; exon 3 encodes the coding region of remaining bigET-1; and the “ET-like peptide” of preproET-1; exon-5 encodes the C-terminal of bigET-1 and the 3’ untranslated region.
Sepsis-induced myocardial dysfunction

Figure 5. Sepsis-induced regulation of ET-1 and G-protein coupled receptor signaling in adult rat ventricular myocytes. Stress/cytokines mediated cascades at cellular and vascular level. It is still not known if ET-1 mediated chronic effects are mediated via activation of PKC-induced p38-MAPK/JNK phosphorylation.

ETs are synthesized from the precursor preproendothelin-1, which is a 212 amino acid peptide (99). After the signal peptide is removed, this propeptide is cleaved by a dibasic amino acid endopeptidase(s) at Arg\(^{52}\)-Cys\(^{53}\) and at Arg\(^{92}\)-Ala\(^{93}\) followed by C-terminal trimming resulting in the formation of a 38-amino acid residue intermediate peptide, termed bigET or proET. The protease involved in the formation of the bigET fragment is a furin-like enzyme. The bigETs are subsequently cleaved at Trp\(^{21}\)-Val\(^{22}\) of ET-1 and ET-2 or at the Trp\(^{21}\)-Ile\(^{22}\) of ET-3 to form the mature peptide (Figure 4) and an inactive C-terminal (22-38) fragment. The final step in the processing of ET is carried out by a membrane-bound zinc metalloprotease, termed ET converting enzyme, ECE-1 (100). The biological significance of this enzyme stems from the fact that the molar potency of bigET was 140 times lower as compared to the mature peptide (101). It is established that preproET-1 does not possess any vasoconstrictor activity (94). The above findings suggest that the conversion of bigET-1 to the mature peptide by the endopeptidase ECE is critical for the marked vasoconstrictor activity of ET-1. Mammalian ET isopeptides mediate their pharmacological actions via binding to their receptors. These receptors belong to the guanine nucleotide-binding protein-coupled receptors (GPCRs) superfamily. Three receptor subtypes for endothelin receptors, ET\(_A\), ET\(_B\), and ET\(_C\), have been identified and cloned (100-104). The ET\(_A\) receptor is predominantly expressed in cardiac myocytes but not in endothelial cells (105-107) and primarily mediates the vasoconstrictor action of endothelins. ET\(_B\) receptors are mostly expressed in endothelial cells and in vascular smooth muscle cells. ET\(_C\) receptor is identified, cloned and characterized from *Xenopus laevis* (104) but not from humans.

4.2.2. ET-1-induced signal transduction

Binding of ET-1 to its receptors induces G-protein coupled stimulation of phospholipase C. The ET\(_A\) receptor may couple to G\(_{i/o}\) or G\(_{q}\) subtypes of G-proteins. G\(_{i}\) consists of an \(\alpha\)-subunit and a member of \(\beta\) and \(\gamma\)-subunit family. The inactive G\(_{i}\) heterotrimer has the \(\alpha\)q ligated to GDP. The activation of GPCRs on binding by ET-1 or an agonist stimulates exchange of GDP for GTP on \(\alpha\)q, causing the heterotrimer to dissociate into \(\alpha\)q (GTP) and \(\beta\)\(\gamma\) dimers (108). This dissociation leads to the activation of phosphoinositide-specific phospholipase C\(\beta\) (PI-PLC\(\beta\)) (109). The PI-PLC\(\beta\) hydrolyses the membrane phospholipids, phosphatidylinositol 4', 5'-biphosphate into two “second messengers”: diaclylglycerol (DAG) and inositol 1', 4', 5'-triphosphate [IP\(_3\)]. These two second messengers have been detected within seconds of exposure of myocytes to ET-1 (110) (Figure 5). Activation of PI-PLC\(\beta\) is terminated by the innate activity of the \(\alpha\)q (GTP) subunits. This innate activity is itself stimulated by the GTPase activating proteins (GAPs) (111). IP\(_3\) diffuses into
Sepsis-induced myocardial dysfunction

4.2.3. ET-1 and sepsis-induced myocardial Dysfunction

ET-1 is implicated in the regulation of regional blood flow and maintenance of vascular tone (84). ET-1 plays an important role in the pathogenesis of various diseases, including myocardial infarction (112), bronchial asthma (113), pulmonary hypertension (114), renal failure (115), and sepsis (13). Sharma et al (13), have demonstrated elevated plasma and myocardial ET-1 levels at 4, 8, 12-h post-sepsis induction that returned to baseline values 24-h later (Figure 6). In another study, we demonstrated that induction of sepsis produced early symptoms of CHF, as evident by elevated tau and increased myocardial work load, oxygen consumption, and LVEDP (57). In one study, we have used a physiological concentration of bigET-1 (which possesses less than one hundredth of the activity of ET-1 as a vasoconstrictor) to double bigET-1 levels in sham and sepsis, in vivo (44). This approach was used to determine if elevated ET-1(1-21) during early sepsis (13) initiates a protective or deteriorative physiological response. We found an elevated myocardial ET-1 concentration in the animals treated with bigET-1 without any significant elevation in plasma ET-1 concentrations in any of the groups studied. This suggests that increased levels of bigET-1 at an earlier time point may be triggering a higher expression of the ET-1 gene leading to mature ET-1 generation in the myocardium. BigET-1 treatment produced similar myocardial dysfunction (as evident by increased tau and LVEDP) both in sham and septic animals (57). This further supports our contention that an elevation of ET-1 in response to sepsis can be an important mediator affecting myocardial dysfunction seen later.

In ARVM, exogenous administration of the ET-1 precursor, bigET-1, elevated the concentration of ET-1 in supernatants and produced hypertrophy both in sham and sepsis groups. In addition, bigET-1 (100 nM) elevated ET-1 by 22 pg/g (~3 fold) in sham group vs. 16 pg/g (~6 fold) in sepsis group (116). Both endogenous ET-1 biosynthesis and exogenous availability of bigET-1 may account for the observed increase of ET-1 concentration in both sham and sepsis groups. Surprisingly, septic ARVM themselves have less basal ET-1. We speculate that this decreased ET-1 concentration in septic ARVM could be due to depressed intrinsic ECE-1 activity, reduced preproET-1 mRNA, or alterations in pretranscriptional regulation of the ET-1 gene. BigET-1 exerted a positive inotropic effect in sham ARVM and up to 3 hours in septic ARVM. However, septic ARVM did not exhibit any further alterations in PS, +dL/dt and –dL/dt following treatment of bigET-1 at 24 h post treatment. This non-responsive effect of the otherwise positive inotrope bigET-1 could be due to activation of various signaling molecules such as p38-MAPK or caspase-3 that have a negative inotropic influence on cardiomyocytes.

4.3. Signaling Cascade

4.3.1. Mitogen-activated protein kinases

Mammalian cells recognize and respond to extracellular stimuli via specific signaling cascades that activate mitogen activated-protein kinases (MAPKs), causing specific cellular responses. MAPKs are implicated in a gamut of cellular events such as mitosis, cell survival, apoptosis, cell differentiation, proliferation, etc. To date, five members of the MAPK family have been characterized in mammals: extracellular signal-regulated kinase1/2 (ERK1/2), p38-MAPK, c-Jun N-terminal kinase (JNK), big MAPK1 (ERK5), and ERKs 3 and 4 (117, 118). However, the most extensively studied MAPK members include ERK1/2, JNK, and p38-MAPK. A classification of the MAPK members has been developed by Philip Cohen, which provides a logical nomenclature for the various members of the MAPK family (Table 2). The MAPKs can be activated by a group of diverse stimuli. In general, the ERKs are predominantly activated by mitogenic and proliferative stimuli such as growth factors and hormones,
ERK1/2 cascade is comprised of the MAPKKs: A-raf, B-raf and most abundantly expressed isoforms (122-124). The ERK1 and ERK2, also known as p44-MAPK and p42-MAPK, pathway. There are five members in this family: ERK1-5. 4.3.1.1. ERK1/2 components (121).

ERK1/2 cascade are involved in the activation and function of the MAPK superfamily. Scaffold proteins bind the existence of additional mammalian MAPKs cannot be ruled out. Although each member of the MAPK superfamily has individual characteristics, several of the following common features are shared by all the members:

1. MAPK cascades are comprised of three conserved, sequentially acting protein kinases: MAPK, MAPK kinase (MAPKK or MKK) and MAPK kinase kinase (MAPKKK) (Figure 7).

2. MAPKs are activated by the dual phosphorylation of Thr and Tyr residues in a ‘T-Xaa-Y’ motif (where Xaa= Glu for ERKs, Pro for JNK and Gly for p38-MAPK) by dual specific MAPKKs. The MAPKKs are themselves activated by MAPKKKs (Table 2).

3. MAPKKKs are serine/threonine kinases and are activated via phosphorylation of small GTP-binding proteins of the Ras/Rho family.

4. Once activated, MAPKS phosphorylate Ser-/Thr residues in target substrates within a (Ser-/Thr-) Pro consensus motif.

5. The MAPK cascade organization is also mediated by interaction with scaffolding proteins. Scaffold proteins bind and sequester selective MAPK components, which coordinate the activation and function of the MAPK components (121).

### Table 2. MAPK nomenclature

<table>
<thead>
<tr>
<th>MAPK</th>
<th>Cohen terminology</th>
<th>Phosphorylation motif</th>
<th>Alternate terminology</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERK1 (44 kDa)</td>
<td>MAPK1</td>
<td>Thr – Glu - Tyr</td>
<td>p44-MAPK</td>
<td>MAPKAPK, MNKs, MKs, Elk1</td>
</tr>
<tr>
<td>ERK2 (42 kDa)</td>
<td>MAPK2</td>
<td>Thr – Glu - Tyr</td>
<td>p42-MAPK</td>
<td>MAPKAPK, MNKs, MKs, Elk1</td>
</tr>
<tr>
<td>JNK (46 or ~54 kDa)</td>
<td>SAPK1a</td>
<td>Thr – Pro - Tyr</td>
<td>JNK2, SAPK-alpha</td>
<td>c-Jun, Jun D, ATF2, Elk1</td>
</tr>
<tr>
<td>JNK (46 or ~54 kDa)</td>
<td>SAPK1b</td>
<td>Thr – Pro - Tyr</td>
<td>JNK3, SAPK-beta</td>
<td>c-Jun, Jun D, ATF2, Elk1</td>
</tr>
<tr>
<td>JNK (46 or ~54 kDa)</td>
<td>SAPK1c</td>
<td>Thr – Pro - Tyr</td>
<td>JNK1, SAPK-gamma</td>
<td>c-Jun, Jun D, ATF2, Elk1</td>
</tr>
<tr>
<td>p38-MAPK (38 kDa)</td>
<td>SAPK2a</td>
<td>Thr – Gly - Tyr</td>
<td>p38-MAPK-alptha</td>
<td>MAPKAPK2/3, MKs, ATF2, Elk1, MEF2C</td>
</tr>
<tr>
<td>p38-MAPK (38 kDa)</td>
<td>SAPK2b</td>
<td>Thr – Gly - Tyr</td>
<td>MAPKAPK2/3, MKs, ATF2</td>
<td></td>
</tr>
<tr>
<td>p38-MAPK (38 kDa)</td>
<td>SAPK3</td>
<td>Thr – Gly - Tyr</td>
<td>ERK6</td>
<td>ATF-2</td>
</tr>
<tr>
<td>p38-MAPK (38 kDa)</td>
<td>SAPK4</td>
<td>Thr – Gly - Tyr</td>
<td>ERK5, BMK, 1</td>
<td></td>
</tr>
<tr>
<td>“Big” MAPK</td>
<td>SAPK5</td>
<td>Thr – Glu - Tyr</td>
<td>ERK5, BMK, 1</td>
<td></td>
</tr>
</tbody>
</table>

MAPK, Mitogen-activated protein kinase; SAPK, Stress-activated protein kinase; JNK, c-Jun N-terminal kinase; Elk, ERK, Extracellular signal-regulated kinase; MAPKAPK, MAPK-activated protein kinase; ATF, Activating transcription factor; MNK, Mitogen-integrating kinase; MEK, MAPK kinase; MSK, Mitogen and stress activated protein kinases; MEF, MEK-enhancing factor; BMK, bigMAPK-1; Thr, Threonine; Glu, Glutamate; Tyr, Tyrosine; Pro, Proline; Gly, Glycine

while p38-MAPK and JNK regulate the cellular response to stress including ultraviolet light, osmotic stress, heat and inflammatory cytokines (119). The MAPK family is highly conserved in all eukaryotes (117). Six diverse MAPKs have been identified in Saccharomyces cerevisiae (120). Thus, the possibility of the existence of additional mammalian MAPKs cannot be ruled out. Although each member of the MAPK superfamily has individual characteristics, several of the following common features are shared by all the members:

1. MAPK cascades are comprised of three conserved, sequentially acting protein kinases: MAPK, MAPK kinase (MAPKK or MKK) and MAPK kinase kinase (MAPKKK or MKKK) (Figure 7).

2. MAPKs are activated by the dual phosphorylation of Thr and Tyr residues in a ‘T-Xaa-Y’ motif (where Xaa= Glu for ERKs, Pro for JNK and Gly for p38-MAPK) by dual specific MAPKKs. The MAPKKs are themselves activated by MAPKKKs (Table 2).

3. MAPKKKs are serine/threonine kinases and are activated via phosphorylation of small GTP-binding proteins of the Ras/Rho family.

4. Once activated, MAPKS phosphorylate Ser-/Thr residues in target substrates within a (Ser-/Thr-) Pro consensus motif.

5. The MAPK cascade organization is also mediated by interaction with scaffolding proteins. Scaffold proteins bind and sequester selective MAPK components, which coordinate the activation and function of the MAPK components (121).

4.3.1.1. ERK1/2

The ERK1/2 cascade is the prototypical MAPK pathway. There are five members in this family: ERK1-5. ERK1 and ERK2, also known as p44-MAPK and p42-MAPK respectively, are the most widely studied MAPKs and most abundantly expressed isoforms (122-124). The ERK1/2 cascade is comprised of the MAPKKKs: A-raf, B-raf and Raf-1; MAPKKs: MEK1 and MEK2; and MAPKs: ERK1 and ERK2. ERK1/2 are potently activated by growth factors (fibroblast growth factor; FGF), phorbol esters (phorbol 12-myristate 13-acetate; PMA), ET-1 and alpha-adrenergic agonists (125). The knockouts for only ERK1 are well characterized. ERK1−/− mice appear normal and are viable with a modest defect in T-cell development (126). This suggests that the functions of ERK1 are conserved by ERK2. MEK1−/− mice die in utero exhibiting defective placental vascularization (127).

In cardiac myocytes, the ERK family of proteins is implicated in survival signaling in response to a horde of stimuli (124). It has also been postulated as a protective signaling mechanism against apoptosis (124). Mitogenic stimuli, such as growth factors, stimulate cell surface receptors, such as tyrosine kinases, that lead to the activation of small 21-kDa guanine nucleotide binding proteins (G proteins) Ras. The members of the Ras family include Ha-Ras, Ki-Ras (Ki-Ras4A and Ki-Ras4B), and N-Ras. Ras is localized to the plasma membrane due to lipid modifications such as farnesylation, carboxymethylation, and palmitoylation. Activation of Ras (Ras.GTP → Ras.GDP) involves stimulation of Sos, a guanine nucleotide exchange factor (GEF) (128). The activated Ras binds to c-Raf, causing its translocation to the membrane, where it gets activated. Activated Raf phosphorylates MEK1/2, which further activates ERK1/2. The precise mechanism of c-Raf activation is still controversial. Nevertheless, activation of c-Raf is crucial for the activation of ERK1/2. Activation of ERK1/2 is also mediated by GPCR agonists such as ET-1. The mechanism may involve DAG-sensitive PKC signaling. Peter Sugden (128) has suggested that PKCs activate Ras followed by c-Raf activation. However, additional receptors for DAG have been identified such as Ras,GRP (a GEF for Ras). Thus, Ras,GRPs may provide a PKC-independent mechanism for Ras activation in the ERK1/2 cascade.

It is now established that ERK1/2 regulates the growth response of cells. In cultured cardiac myocytes, ERK has been shown to be protective against apoptosis (129). Various studies suggest a role of ERK1/2 in cardiac myocyte hypertrophy (128). Evidence of compensated biventricular hypertrophy has been observed in transgenic mice, where ERK1/2 was activated by constitutively expressed MKK1. These mice showed improved myocardial contractility with resistance towards induction of apoptosis (130). Further evidence that demonstrated transient activation of ERK1/2 stimulates multiplication,
Sepsis-induced myocardial dysfunction

Figure 7. Various messenger systems involved in signal transduction pathways.

While sustained ERK1/2 activation can cause cell cycle arrest (131). However, the role of ERK 1/2 in the induction of apoptosis is still debatable.

The ERK1/2 cascade is generally associated with cell proliferation. In neuronal cells, sustained ERK1/2 activation by NGF induces differentiation. However, EGF produces transient ERK1/2 activation, ERK1/2 which contributes to the increased proliferative rate of tumor cells (132, 133). ERKs mediate responses in the central nervous system, such as regulation of learning and memory. MEK1 is also essential for the migration of vascular endothelial cells (127). Activation of ERK1/2 also modulates protein kinases (90-kDa ribosomal protein s6 kinases; p90RSKs) (134), transcription factors (Elk-1) (135), and other signaling proteins (phospholipase A2) (136).

In vivo, ERK1/2 activation has been shown to attenuate apoptosis subsequent to ischemia-reperfusion injury of the intact heart (137). In a polymicrobial septic rat model, we did not find any change in myocardial ERK1/2 expression at 24h post-sepsis induction, in vivo (23, 57). However, we observed that hyperglycemia produced an upregulation of ERK1/2 in septic rats (23). In vitro expression of activated ERK1/2 is significantly upregulated in septic ARVMs. However, treatment with bigET-1 did not further increase the expression of activated ERK1/2 in sepsis or in sham (116, 138). This suggests that bigET-1 does not modulate its effects through the ERK pathway in ARVM. Therefore, we concluded that during chronic sepsis, ERK1/2 may not play a significant role in sepsis-induced myocardial dysfunction.

4.3.1.2. p38-MAPK

The second member of the MAPK superfamily, p38-MAPKs, are activated by stress (arsenite, hypoxia/reoxygenation, ROS, hyperosmotic shock), UV, proinflammatory cytokines, and endotoxins (117, 139). The p38-MAPK cascade consists of MAPKKKS: MEKK1-4, MLK2 and –3, DLK, ASK1, Tpl2, and Tak1; MAPKKs: MEK3 (MKK3), and MEK5 (MKK6); MAPK: p38-MAPKs. The mammalian p38-MAPK is homologous to HOG1, the osmosensing MAPK of S. cerevisiae (140).

Several isoforms of p38-MAPK have been characterized: alpha-1/alpha-2 (141), beta-1/beta-2 (142, 143), gamma (144), and delta (145). The isoform p38-gamma is predominantly expressed in skeletal muscle, while p38-delta is widely expressed in various adult tissues and during development (146). p38-MAPK alpha and beta isoforms are more prevalent in the human heart than the other two isoforms. Human p38-alpha was originally purified and cloned as a polypeptide receptor for cytokine-suppressive anti-inflammatory drugs (CSAIDs) (141). Hence, p38-alpha is also termed as a CSAID-binding protein (CSBP). Among the four isoforms, only p38-MAPK alpha and beta are inhibited by CSAIDs, with the gamma and delta isoforms being non-responsive to these drugs (145). The knockout of only p38-MAPK alpha is available. Inactivation of p38-alpha causes extensive embryonic lethality (147). However, the severity and the cause of lethality vary with the genetic background in which p38-alpha deficiency is examined (148). MKK3−/− mice are viable without any obvious abnormalities (149). The macrophages of MKK3−/− mice exhibit reduced p38-MAPK activity by endotoxins (149).
Activation of p38-MAPK results by dual phosphorylation of Thr180 and Tyr182 by the upstream MAPKK, MKK6 and MKK3. MKK6 and MKK3 are in turn activated by several MAPKKks described above in response to stress stimuli. MKK3 and –6 are highly specific for p38-MAPK activation and do not activate ERK1/2 and JNK. MKK6 activates all four isoforms of p38-MAPK, while MKK3 preferentially activates the p38-alpha and - beta isoforms. MKK4, on the other hand, activates JNK along with p38-MAPK, representing an upstream overlapping site for the p38-MAPK and JNK cascades.

Another mechanism has been suggested for the activation of p38-MAPK independent of the prototypic MAPKKs cascade (150). Interaction of p38-alpha with TAB1 (transforming growth factor-beta-activated protein kinase 1 (TAK1)-binding protein 1) produces intramolecular autophosphorylation and activation of p38-alpha. TAB1 is not a MKK and appears to be an adaptor or scaffolding protein with no known catalytic activity.

The biological functions of p38-MAPK include regulation of the immune and inflammatory responses, gene expression, interstitial remodeling, contractility, energy metabolism, and hormone synthesis (151, 152). Inhibition of p38-MAPK has been shown to downregulate the production of cytokines such as TNF-alpha, IL-1, etc. (153, 154). Mounting evidence suggests involvement of p38-MAPK in apoptosis, chemotaxis, granular exocytosis, cell differentiation, etc. (118, 151). Conflicting evidence also suggests an implication of p38-MAPK as a survival factor in contrast to being pro-apoptotic (139). The different isoforms of p38-MAPK also perform distinct biological functions. p38-MAPK alpha is pro-apoptotic (124), while p38-MAPK alpha induces a hypertrophic response. An upregulation of Bcl-2 protein expression in hearts of dominant-negative p38-alpha transgenic mice has been observed, suggesting a potential protective mechanism associated with p38 inhibition (155). Activation of p38-MAPK has also been shown to increase p53 protein levels, which in turn promotes apoptosis by inducing the expression and mitochondrial translocation of Bax (156, 157). Moreover, p38-MAPK is shown to translocate to the mitochondria of neuronal cells in response to nerve growth factor withdrawal, where it directly phosphorylates Bcl-2, inactivating its anti-apoptotic effects (158). p38-MAPK is known to localize to mitochondria in cardiac myocytes (159).

In the myocardium, activation of p38-MAPK has been observed in ischemia, ischemia/reperfusion (160), oxidative stress (139, 161), and heart failure (162, 163) in humans and animal models. MAPKs, particularly p38-MAPK, have been implicated in hypertrophy of cardiac myocytes (164, 165). However, in another study, dominant-negative mutant of p38-MAPK did not affect pressure overload-induced hypertrophy in cardiac myocytes but stimulated resistance to cardiac fibrosis (166). Inhibition of p38-MAPK activity in vivo attenuates cardiac remodelling and heart failure during myocardial infarction (167). Further, p38-MAPK has been shown to mediate a negative inotropic effect without altering intracellular calcium homeostasis in adult rat cardiac myocytes (ARVM) (168). In transgenic mice, p38-MAPK contributed to restrictive cardiomyopathy by induction of fetal gene expression, interstitial fibrosis, and loss of myocardial contractility (169). p38-MAPK also regulates cardiac cyclooxygenase-2 and prostaglandin biosynthesis (170, 171). However, the functions of p38-MAPK at the myocardium level are complex and are less clearly understood due to the presence of the various isoforms with diverse functions. Activation of p38-MAPK alpha in response to hypoxia has been shown to stabilize hypoxia-induced of erythropoietin (Epo) mRNA in human hepatoma cells (162) implicating the involvement of p38-MAPK in development. However, since the genetic knockouts of p38-MAPK alpha are lethal (147), the understanding of p38-MAPK phosphorylation in human pathophysiology is hindered.

Both in vivo and in vitro models of sepsis have shown an upregulation of phosphorylated p38-MAPK in ARVM and left ventricular tissue (57, 116). Treatment with bigET-1 caused an upregulation in p38 MAPK phosphorylation in sham and even further in sepsis. Septic ARVM, which showed depressed contractility, responded briefly to the positive inotropic effect of bigET-1. However, at 24 hours, at which point p38 MAPK phosphorylation is upregulated, septic ARVM did not show a response to bigET-1 (138). By inhibiting p38-MAPK with SB203580, bigET-1 exerted a positive inotropic effect in septic ARVM, but not to the same extent as seen in sham ARVM. This suggests that signaling mechanisms related to contractility are disrupted under septic conditions (138). Upon treatment of ARVM with FR901533, an ECE-1 inhibitor, and bigET-1, no significant change was seen in contractility despite a further upregulation of p38-MAPK phosphorylation (116). In vivo, bigET-1 has been shown to upregulate p38-MAPK phosphorylation in sham but not in sepsis. BigET-1 worsened the already existing myocardial dysfunction in sepsis as reflected by an increase in tau (57). Since p38-MAPK possesses a negative inotropic effect and activated for longer durations, its role in sepsis-induced myocardial dysfunction appears to be critical.

4.3.1.3. JNK

JNKs are another member of the MAPK superfamily. On a broader perspective, JNKs are members of the CMGC family of protein kinases of the human kinome (172). JNKs are also referred to by alternative nomenclature: 1) stress-activated protein kinases, because of their activation in response to stress; and 2) “JNK,” in reference to their ability to phosphorylate the N-terminal of the transcription factor, c-Jun (117). JNKs are potentially activated by stress, proinflammatory cytokines, UV, DNA-damaging agents, growth factors, etc. (117). Stress that activates JNKs can be both cellular and mechanical. Cellular stresses include hyperosmotic shock, low concentrations of protein synthesis inhibitors (anisomycin), hypoxia/reoxygenation, and ROS (139). JNKs are not activated during global ischemia. Rather, their activation occurs during the reperfusion phase (160). Mechanical stresses that activate JNKs are passive stretch and electrical pacing. It is speculated that increasing the wall stress in intact hearts causes release of ET-1 and/or Ang II that
activates JNK (or p38-MAPK) in an autocrine/paracrine fashion (173).

Mammalian JNKs are encoded by three separate genes (JNK1, JNK2, and JNK3) localized on chromosomes 10q11.1-11.2, 5q35.3, and 4q21-g22.1, respectively (172). The three JNKs are further spliced to ten different proteins (174). Although all the JNKs are ubiquitously expressed, JNK3 is predominantly expressed in the brain. All the isoforms, however, differentially recognize and phosphorylate their transcription factor substrates (174). All three JNK loci have been knocked out. JNK1/2-/- die at mid-gestation with defective neural-tube closure (175). Deletion of MKK4 causes abnormal liver development, etc. (152). Wang et al (177) have demonstrated that activation of JNK via expression of activated MKK7 resulted in cardiac myocyte hypertrophy in vitro. Similarly, in cultured neonatal ventricular myocytes inhibiting JNK via expression of inactive MKK4 attenuated ET-1 induced hypertrophy in these myocytes (178). Dominant-negative JNK inhibited PE-induced ANF expression (179). Liang et al (180) have shown attenuation of hypertrophy by JNK in a model of pressure-overload-induced hypertrophy. Also, ASK-1-/- hearts show different effects on hypertrophy. Briefly, Ang II-induced cardiac hypertrophy was attenuated by reduced JNK activation (181). However, in the similar animals JNK reduced hypertrophy by pressure-overload (182). Thus, it appears that JNK may affect cardiac hypertrophy in a disparate manner depending on the nature of the hypertrophic stimuli.

JNK1 inhibition is reported to actually protect cardiac myocytes from ischemia-induced apoptosis, where as JNK2 inhibition had no effect (183). Activation of JNK has been shown to induce apoptosis in ARVM during oxidative stress (184). Similarly, increased apoptosis via JNK has also been observed in UV-irradiated fibroblasts (185). In contrast, a protective role of JNK1 signaling has been suggested during nitric oxide-induced cardiomyocyte apoptosis (186). JNKs have also been shown to attenuate apoptosis induced by nitric oxide (NO) in neonatal myocytes (172). However, the role of JNK signaling in regulation of apoptosis is still controversial.

4.4. Apoptosis cascade

Contrary to the earlier theory that cardiac myocytes perish due to necrosis, there have been numerous studies to establish the programmed death or apoptotic pathway in these vital cells. However, apoptosis in sepsis is still a mystery remaining to be deciphered. Septic myocytes can potentially apoptosis via activation of the death receptor mediated extrinsic pathway or the mitochondrial intrinsic pathway. The extrinsic machinery is a receptor-controlled phenomenon. It involves activation of cell surface death receptors by the tumor necrosis factor superfamily constituting extracellular ligands such as TNF-alpha and Fas ligand. The best characterized death receptors are Fas (CD95), TNF receptor -1 (TNFR1), and TRAIL (TNF-alpha related apoptosis inducing ligand) (187, 188). Since host response in sepsis stimulates proinflammatory cytokines such as TNF-alpha, induction of extrinsic pathway is inevitable. Interaction between the death receptor and ligand initiates the formation of a DISC (death-inducing signaling complex), along with procaspase-8. This results into an autophosphorylation reaction causing activation of downstream caspases such as caspase-3. Caspases possess DNase activity which ultimately results in DNA fragmentation (189). Anti-apoptotic X-linked inhibitor of apoptosis proteins (XIAP) can potentially prevent death receptor mediated apoptosis by binding to and inhibiting caspase-3 (190).

The intrinsic apoptotic cascade is a stress-activated pathway (191). Negative stress signals cause mitochondrial perturbation, resulting in the release of proteins that trigger the activation of caspases. Mitochondrial activity also culminates in disruption of electron transport and alteration of the cellular redox potential (192). Mitochondrial proteins having the greatest consequences are cytochrome C and second-mitochondria-derived activator of caspases (Smac). Once released cytochrome C, with the aid of ATP, induces the oligomerization of Apaf-1 to form the “apoptosome.” This complex recruits the activation of initiator caspase-9, which amplifies the signal to stimulate effector caspases 3, -6, and 7 (193-195). Release of pro-apoptotic Bcl-2 proteins such as Bax promotes the permeabilization and disruption of the mitochondrial membrane (196). However, the cross-talk between the extrinsic and intrinsic pathway involves yet another Bcl-2 protein, Bid. Active caspase-8 can cleave this protein and thus cause its translocation from the cytosol to the outer mitochondrial membrane, stimulating the release of cytochrome C. Previously we demonstrated that sepsis upregulates p38-MAPK phosphorylation. This increase correlates with the activation of caspase-3, as seen in septic cardiomyocytes at 12 and 24 hr. We have also demonstrated that bigET-1 causes an increase in caspase-3 activity at 24 h in sham and septic myocytes (138). This increase in caspase-3 activity was not altered following treatment with a cell permeable caspase-3 inhibitor Ac-DEVD-CHO probably due to reversible inhibition of this drug. Also, treatment of the cells with captopril, a selective angiotensin converting enzyme inhibitor, did not alter caspase-3 activity (116).

These findings can also reflect the involvement of the extrinsic apoptosis pathway in septic myocytes. Our findings also corroborate that p38-MAPK exerts pro-apoptotic effects, as suggested by Gonzalez et al (197). ASK-1 is a member of the MAPK family that activates JNK & p38-MAPK and executes apoptosis by mitochondria dependent caspase-3 activation, which involves the phosphorylation of Bcl2 along with the release....
Sepsis-induced myocardial dysfunction

of cytochrome C (198). ATF-2, a downstream effector in the MAPK cascade that is a member of the ATF/cAMP response element binding protein family, also plays an important role in cellular stress response (199). Activation of p38-MAPK mediated upregulation of Bax, Bcl2, and activation of caspase-3 has been reported as one of the main pathways for apoptosis and hypertrophy in ARVM (200). Activation of the innate immune system following sepsis initiates the release of pro-inflammatory cytokines, which act on TLRs (toll like receptors). NF-kB is the central downstream target of the TLR-mediated pathway. NF-kB related genes have been implicated in the pathogenesis of cardiac dysfunction in sepsis. Several MAPKs including JNK and p38-MAPK also cause phosphorylation of NF-kB.

4.4.1. Role of calcineurin

Calcineurin (PP2B) is a serine/threonine protein phosphatase that responds to elevated intracellular calcium (201-203). On activation, calcineurin dephosphorylates members of the NFAT transcription factors in the cytoplasm. Dephosphorylated NFAT translocates to the nucleus, where it has been shown to play an important role in regulating the hypertrophic growth response (204). Calcineurin’s role in apoptosis is controversial as in some cell types it agonizes, while in others it antagonizes apoptosis as reported (124). Calcineurin is reported to function in a way that its transient activation antagonizes myocytes apoptosis. However, its prolonged activation induces cardiac hypertrophy and deleterious effect on the heart (124). Calcineurin has been proposed to play a role in the activation of iNOS in LPS-stimulated mouse peritoneal macrophages, but its role in chronic sepsis-induced myocardial dysfunction in vivo is not known (205).

4.4.2. Protein kinase C (PKC) signaling

PKC is a family of 11 serine/threonine kinases, most of which have been identified in the heart (206-208). These 11 enzymes are subdivided into three groups: classical PKCs are those which are activated in presence of Ca2+ and DAG; novel PKCs that are activated by only DAG; and atypical PKCs which are insensitive to Ca2+ or DAG but are activated by certain lipids (206). Classically, G-protein coupled receptors (GPCRs) activate PKC, which in turn hydrolizes phospholipids and elevates intracellular DAG and calcium (206). However, PKC activation can also occur through growth factor receptors, nitric oxide and reactive oxygen species in the myocardium (209-211). PKCδ and PKCε have distinct effects on cardiac myocyte survival and death (124). In cardiac myocytes PKCδ has been found to selectively activate the JNK and p38-MAPK pathways, whereas PKCe activates the ERK pathway (212). Cardioprotection mediated through PKCδ has been shown to be associated with an anti-apoptotic kinase Akt (213). Translocation of PKCδ to mitochondria is essential for initiation of mitochondrial death pathway (214-217). PKCe, on the other hand, can prevent mitochondria-mediated cell death (218, 219). In our earlier study, we could not observe any alteration in the expression of myocardial PKCs at 24-h post-LPS administration, in vivo (220). It appears that the activation of PKC-ε can play a role during very early activation of sepsis-induced signaling molecules or mediators. However, its precise role needs to be elucidated further.

Other regulatory kinases like protein kinase A (PKA) and calcium/calmodulin kinase (CaMK) have been shown to play a role in myocardial apoptosis (124). However, their specific roles during sepsis-induced myocardial dysfunction are far from clear.

5. FUTURE PROSPECTIVES

The data from our group demonstrate that ET-1 precursor produces decompensatory hypertrophy in ventricular myocytes and contractile dysfunction (hyporesponsiveness to bigET-1). The role of ET-1 on mitochondrial oxidation cascade and extrinsic apoptosis during sepsis is not yet clear. We suspect that poor understanding of myocardial signaling during early sepsis could be one of the main reasons for limited success of pharmacotherapeutic options for sepsis. It appears that signaling cascade activation, apoptosis, and contractility alterations during sepsis needs to be thoroughly elucidated so as to limit the myocardial damage caused by sepsis, septic shock, and related cardiovascular disorders.

6. ACKNOWLEDGMENTS

The research work in Dr. Sharma’s laboratory is supported by NIH, HL066016, American Heart Association Greater Midwest Beginning Grant-In-Aid (AHA#0160444Z). SB received a Predoctoral fellowship from American Heart Association Greater Midwest affiliate (AHA#0510075Z) and AG received a Presidential fellowship from North Dakota State University.

7. REFERENCES

Sepsis-induced myocardial dysfunction

39. Parker, M. M., K. E. McCarthy, F. P. Ognibene & J. E. Parrillo: Right ventricular dysfunction and dilatation, similar to left ventricular changes, characterize the cardiac depression of septic shock in humans. Chest 97, 126-31 (1990)
Sepsis-induced myocardial dysfunction


Sepsis-induced myocardial dysfunction

can predict the development of multiple organ failure in patients at risk. *Crit Care Med* 24, 392-7 (1996)
Sepsis-induced myocardial dysfunction


Sepsis-induced myocardial dysfunction


144. Li, Z., Y. Jiang, R. J. Ulevitch & J. Han: The primary structure of p38 gamma: a new member of the p38 group of MAP kinases. *Biochem Biophys Res Commun* 228, 334-40 (1996)


204. Wilkins, B. J. & J. D. Molkentin: Calcineurin and cardiac hypertrophy: where have we been? Where are we going? *J Physiol* 541, 1-8 (2002)


Sepsis-induced myocardial dysfunction


**Key Words:** Inflammation, Infection, Sepsis, Systemic Inflammatory Response Syndrome, Peritonitis, Polymicrobial sepsis, tau, Apoptosis, Review

**Send correspondence to:** Avadhesh C Sharma, PharmD, PhD, FAHA, Cardionome Laboratory, Department of Pharmaceutical Sciences, North Dakota State University, 208 Sudro Hall, Fargo, ND 58105, Tel: 701-231-7780, Fax: 701-231-8333, E-mail: Avadhesh.sharma@ndsu.edu

http://www.bioscience.org/current/vol10.htm