REVIEW ARTICLE

The role of endothelium and endogenous vasoactive substances in sepsis

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Abstract

Sepsis and septic shock are great challenges for the doctors who treat critically ill patients. A big part of the scientific community is performing researches about the pathophysiology and treatment of this clinical problem. The endothelium has a very significant role in the alterations that sepsis causes especially to the circulatory system. The disorders of the normal function of the endothelium include derangement of the vascular tone, increase of endothelium permeability, activation of the endothelial cells, production of various regulators and disorders of coagulation. Nitric oxide is the modulator that mediates the action of most vasodilators. The overproduction of nitric oxide during sepsis is possibly the most important cause of the vasopressor-resistant hypotension which characterizes septic shock. The levels of natriuretic peptides are also increased. These peptides act through several ways on the circulatory system both peripherally and directly on the myocardium. Endothelin, vasopressin, adrenomedullin and prostacyclin are vasoactive substances that have their own role in the regulation of the circulatory system during sepsis. Hippokratia 2010; 14 (2): 88-93

Key words: Ssepsis, endothelium, nitric oxide, endothelin, natriuretic peptides, vasoasctive substances

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Inflammation, sepsis and septic shock are the greatest challenges for the intensivist. Despite the important advances in intensive medicine, the morbidity of sepsis remains very high. According to Angus et al[1] the morbidity of sepsis in the Intensive Care Unit is about 28.6% and the cost for the treatment of every patient is 22,100$.

Definition of sepsis

Sepsis is the systemic inflammatory response to bacterial inflammation[2]. Sepsis is one type of the Systemic Inflammatory Response Syndrome(SIRS) which can be triggered by other causes besides inflammation like trauma, serious burn, major surgery etc[3]. When the inflammatory response is expressed as dysfunction of one vital organ (heart, liver, lung, kidney), sepsis is characterised as serious, and when two or more organs are involved the situation is called Multiple Organ Dysfunction Syndrome (MODS). When a patient with sepsis develops acute circulatory failure (systolic blood pressure<90mmHg, mean arterial pressure<70mmHg or 40mmHg below the normal values despite adequate intravenous fluid therapy) the clinical condition is defined as septic shock[2]. The diagnostic criteria of sepsis[4] are summarised in Table 1.

The pathophysiology of sepsis is under constant research. Over the last years there has been great progress in understanding the complicate mechanisms which are involved in the beginning, the course and the final outcome of sepsis. Despite the progress, there are questions that have not been answered yet. Lipopolysaccharides of the bacterial cellular wall have an important role in the beginning of the inflammatory cascade because they activate the inflammatory cells and the cells that participate in the coagulation

Table 1: Diagnostic criteria of sepsis

Inflammation documented or suspected and some of the above
General parameters
Fever (Temperature >38.3° C)
Hypothermia (Temperature <36° C)
Heart rate >90/min or >2 standard deviations from normal for the age
Tachypnea
Disorders of conscience
Edema or positive fluid balance (>20 ml/kg/24h)
Hyperglycemia (plasma glucose > 120mg/dl) with no history of diabetes mellitus
Inflammatory parameters
Leucocytosis(White blood cells >12000/μL)
Leucopenia (White blood cells <4000/μL)
Normal blood cells with >10% immature cells
CRP >2 standard deviations
Procalcitonin >2 standard deviations
Hemodynamic parameters
Hypotention (Systolic blood pressure <90mmHg, Mean arterial pressure <70mmHg, fall of blood pressure >40mmHg or >2 standard deviations from normal for the age)
SvO_2 > 70%
Cardiac index >3.5L/min/m^2
Organ dysfunction parameters
Hypoxemia (PaO_2/FiO_2>300)
Oliguria(urine <0.5ml/kg/h)
Blood Creatinine >0.5mg/dl
Coagulation disorders (INR>1.5 or aPTT>60s)
Ileum
Thrombocytopenia (platelets <100000/μl)
Bilirubin >4mg/dl)
Tissue perfusion parameters
Lactate >1mmol/L)
Reduced capillary refill

mechanism[2]. The response of the host, which follows, is the main factor that determines the outcome of sepsis and is expressed through the action of the monocytes and endothelial cells[5]. The production of various cytokines and inflammatory mediators and the activation of the coagulation cascade are caused by these cells and are the main causes of multiple organ dysfunction. Recently, the role of high mobility group box 1 (HMGB-1) nuclear protein in sepsis has been studied. This protein is released into the circulation after the death of cells or it is produced and secreted by macrophages and endothelial cells due to stimulation by inflammation. It is considered to be a major regulator of inflammation and to affect the outcome of sepsis[6].

The dysfunction of the circulatory system due to sepsis greatly affects morbidity which can be up to 90% in cases of septic shock[7]. The reaction of the circulatory system to sepsis is characterised by an initial hyperdynamic phase, where a very big reduction of the systemic vascular resistance is observed due to peripheral vasodilation unresponsive to vasoconstrictive drugs and the myocardial dysfunction is not obvious because of the increase of cardiac output and stroke volume[8]. Later the myocardial dysfunction caused by the inflammatory mediators[9] leads to the hypodynamic phase where cardiac output falls and both the systolic and the diastolic function of the heart are greatly impaired[10]. The endothelium and the various vasoactive substances like nitric oxide (NO), natriuretic peptides, endothelins, vasopressin, adrenomedullin, and prostacyclin have a very important role in the mechanisms that affect the circulatory system during sepsis.

The role of endothelium in sepsis

The endothelium, although it is not anatomically described as an individual organ, is one of the most important human organs. It is consisted of about 10^{11} cells, its weight is 1kg and the area which covers is 4000 – 7000 $m^{2,5}$. The endothelium performs some very important actions like regulation of the vascular tone, control of the circulation of nutrients between intravascular and extravascular space and preservation of the blood flow by regulation of coagulation[12]. Moreover it participates in the physiology of atheromatosis, autoimmune disorders, inflammation and angiogenesis[13]. The reaction of endothelium to various stimuli is dynamic and differs according to the kind of the stimuli, the place of the vascular tree where the stimuli act and the time (moment and duration) of the stimuli[11].

Concerning inflammation, its effect on the endothelium is characterised by damage of the endothelial cells, disorder of their normal function, activation and death via apoptosis[14]. The damage at cellular level can vary in type (swelling and destruction of the cytoplasm, nuclear disorders etc) but the final result is detachment of the endothelial cells from the vascular wall, due to subendothelial edema in the place of the damage, and disorder of the permeability of endothelium[15]. Derangement of the normal function includes disorder of the vascular tone[16] while activation of the endothelial cells includes changes in the expression of various proteins of the membrane[14]. Finally apoptosis is the procedure through which certain intracellular or extracellular signals activate mechanisms that damage the DNA of the nucleus and is believed to be triggered by sepsis[17]. The inflammatory mediators (TNF-a, IL-1, free oxygen radicals etc) and the activated monocytes induce programmed cell death[5] by activating caspaces which cause the lysis of the endothelial cell[18]. The apoptotic endothelial cells induce the inflammatory reaction by producing cytokines and free oxygen radicals and activating the complement system[5].

Regarding coagulation, cytokines, like TNF and IL-1, which are secreted by the inflammatory cells, act on the endothelial cells causing inhibition of the production of thrombomodulin, antithrombin-III, tissue plasminogen activator and heparan sulphate[5]. The result of these alterations is the blockade of plasmin formation and fibrinolysis. On the other hand the activated endothelial cells overexpress the tissue factor and the plasminogen activator inhibitor triggering the exogenous coagulation cascade[19]. The activated endothelial cells affect the coagulation mechanism by another way too. They secrete the von Willebrant factor[20], which is very important for the adhesion and activation of platelets, and they overexpress on the cellular membrane agglutination proteins like E and P selectins[21] which stimulate the adhesion of platelets and monocytes on the endothelium. The consequence of these disorders is the formation of microthrombi which are able to alter the microcirculation of the capillaries and the perfusion of the vital organs. This disorder is responsible for various complications of sepsis like ARDS (Adult Respiratory Distress Syndrome), myocardial and intestine dysfunction, hepatic and renal failure and DIC (Diffuse Intravascular Coagulation)[14].

The results of sepsis on the endothelium regulated vascular permeability are critical. Various inflammatory mediators like TNF, IL-1 and INFγ cause remodeling of the cytoskeleton of the endothelial cells[22]. Moreover, the response of the endothelial cells to cytokines frequently is combined with an increase of intracellular Ca^{2+} which is related to contraction and separation of the cells[23]. Also, some cells of the endothelium, activated by oxidative stress, produce reactive oxygen species (ROS) which destroy other endothelial cells[24]. The result of all the above alterations at cellular level is the disruption of the endothelial barrier and the extravasation of liquid and proteins in the interstitial space, known as capillary leak. The increased production of antithrombin, which is mentioned above, aggravates the capillary leak[25]. The leakage of intravascular fluid causes the intravascular volume to gradually shrinking, which is very resistant to intravenous fluid administration, and peripheral edema which is characteristic of sepsis[26]. Finally the role of capillary leak in ARDS and SIRS, which can follow sepsis, is crucial[27].

The major pathophysiologic characteristic of sepsis and mainly septic shock is the loss of control of the vascular tone. The balance between vasoconstrictors and vasodilators is greatly disrupted[28]. Although the endothelium produces both substances, the action of vasodilators finally dominates and the peripheral systemic resistance decreases dramatically. Also endotoxemia directly de-

presses the postsynaptic sympathetic nerve fibers which innervate vascular smooth muscle fibers and downregulates the baroreflex function[29]. The vasodilation is very resistant to exogenous vasopressors like epinephrine and norepinephrine[30]. The cardiac output initially rises, as a response to vasodilation, but finally the circulatory system collapses and the cardiac output seriously drops. The circulatory failure is accompanied by microcirculation disorder of the organs and the result is inadequate oxygen delivery and finally failure of the vital organs[31]. The role of various vasoactive substances, whose production, secretion and action are seriously impaired during sepsis is very important in vascular tone modulation.

The role of endogenous yasoactive substances in sepsis

Nitric oxide

Nitric oxide (NO) is a substance produced by endothelial cells after their stimulation by acetylcholine and causes vasodilation[32]. Apart from endothelial cells, NO is secreted by other cells (like macrophages) and acts as a neurotransmitter too[33]. NO is produced from the amino-acid L-arginine by the action of the enzyme nitric oxide synthase (NOS) which comprises three isoenzymes. The two of the isoenzymes are expressed in the endothelium (endothelial NOS or eNOS) and the nervous cells (neuronic NOS or nNOS) while the function of the third isoenzyme (induced NOS or iNOS) is induced by various cells (macrophages, hepatocytes, smooth muscle cells, myocardial cells etc.) and products of Gram positive and negative bacteria[34]. The first two isoenzymes are commonly referred as constitutive NOS or cNOS. The main difference between cNOS and iNOS is that activation of cNOS is depended on the calcium-calmodulin system, lasts shortly and causes the production of small amounts of NO while iNOS activation lasts longer and causes the production of significantly larger amounts of NO[35].

The action of NO is performed by modulation of cGMP production which via a series of complicated biochemical procedures reduces intracellular Ca^{2+} and causes the relaxation of the vascular smooth muscle fibers and vasodilation[34,35]. As a result the role of NO in the regulation of blood pressure and organ blood flow is very important[36]. The production of NO is stimulated by other substances apart from acetylcholine like bradykinin, histamine, serotonin, and free fatty acids while the endothelial cells themselves act as mechanoreceptors and in cases of peripheral alterations of blood flow and viscosity they generate signals for the production of NO[37]. Apart from the regulation of vascular tone, NO inhibits the adhesion of platelets and white blood cells on the endothelium and the production of superoxide anions by them[38-40]. Although NO neutralises the free toxic radicals (like O_2^-), in some cases, like sepsis, it participates in the reactions that produce toxic products[34]. The inhibition of NO production is done either by feedback regulation of NOS or by L-arginine analogs.

During sepsis the role of NO in vasodilation, vascular permeability disorder and aggregation and activation of white blood cells and platelets is critical. The most important factor that causes the cardiac output independent vasodilation, which is very resistant to exogenous catecholamines (vasoplegia), is the overproduction of NO. The cause of NO overproduction is the induction of iNOS enzyme. The endotoxins and the inflammatory mediators like IL-1, IL-2, IL-6, TNF, INF-γ and others, have been proved to induce this enzyme in vitro[41]. The induction of iNOS can last for several days after the initial stimulation. The fact that the overproduction of NO is attributed to iNOS and not eNOS induction partly explains the lack of response to administration of noradrenaline. This was studied by Flemming et al[42] when they observed that sepsis vasodilation occurs even at vessels without endothelium while noradrenaline resistance stops when the production pathway of NO from arginine is inhibited. Other mechanisms responsible for NO overproduction are the increase of available arginine and the induction of cNOS enzyme whose contribution is minimal[43]. Besides vasodilation NO participates in cell destruction by reacting with ROS and producing toxic substances (like ONOO)[21]. Finally NO appears to directly depress the function of myocardial cells during sepsis, contributing to general myocardial dysfunction[44]. The possible explanation for this fact is that the high levels of cGMP that follow NO overproduction decrease the flow of calcium ions in the sarcoplasma of myocardial cells and the response of myocardial muscle fibres to calcium[34].

The inhibition of NOS and the reduction of NO levels appear to have significant clinical results. A 72-hour treatment with the NOS inhibitor 546C88 reduced the plasma levels of NO in patients with diagnosed septic shock. The result was the upregulation of the vascular tone, the decrease of cardiac index and oxygen delivery and the maintenance of mean arterial pressure over 70mm Hg with less doses of inotrope drugs compared to the septic patients who were taking placebo[45]. Finally inhibition of NOS promoted the resolution of shock in patients with severe sepsis[46].

Natriuretic peptides

The brain natriuretic peptide (BNP), the atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) have been recently identified as important factors of circulatory regulation. BNP is produced mainly by the myocardial cells of the ventricles[47] initially as prohormone (proBNP) which is cleaved to active BNP and inactive NT-proBNP. The basic stimuli for BNP production is the increased tension of the ventricular muscle fibres mainly due to volume overload[48]. BNP increases glomelular filtration by dilating the afferent and contracting the efferent arteriole, increases sodium excretion in the urine, inhibits aldosterone action, decreases systemic blood pressure via peripheral vasodilation, decreases the preload and inhibits the sympathetic nervous system[49]. ANP is produced by atrial cells which respond to the same stimuli that cause the production of BNP from the ventricles (increased tension and volume in the atria). It is also produced as a prohormone and causes systemic blood pressure drop by vasodilation and increase of di-

uresis and sodium and potassium loss in the urine[3]. CNP has been localized in the endothelial cells of various vessels and in the systemic circulation and its main role is not natriuresis like, the other natriuretic peptides, but endothelium independent vasodilation mainly of the coronary arteries[50]. The action of these peptides on the periphery is performed by the stimulation of their receptors NPR-A, NPR-B and NPR-C. NPR-A is related to the action of ANP and BNP, NPR-B to the action of CNP while NPR-C is a clearance receptor related to the removal of the peptides from circulation[3].

In sepsis the plasma levels of ANP and BNP are significantly increased[51,52]. Disorder of normal cardiac function could possibly be the reason. The reduction of ejection fraction in septic patients is trying to be balanced by an increase of end-diastolic volume of the left ventricle[53] which is a stimulus for the production of BNP. But in a number of studies the plasma levels of BNP and NT-proBNP did not correlate to the volumes of the ventricles of septic patients nor to the pulmonary capillary wedge pressure[52,54,55]. There are suggestions that sepsis causes a direct depression of myocardial function and ventricle dilatation (not caused by the end-diastolic volume)[56] but this fact alone cannot explain the high levels of BNP. Endotoxins, IL-6, and other proinflammatory cytokines have been proved to produce direct stimulant substances for the production of BNP and ANP, independent from cardiac function, in animal[3] and human studies[57]. Furthermore it seems that this mechanism is mainly responsible for the production of ANP during sepsis and not the increase of pressure in the atria[51]. Vasoconstrictor substances like angiotensin and endothelin, which are overproduced in sepsis, also stimulate the production of BNP[58]. Recent data prove that the relation between BNP and sepsis is much more complicated. BNP seems to play an important role in the regulation of inflammatory mediators' production. Substances, like reactive oxygen and nitrogen species (ROS and RNS), leukotriene B_4 (LTB4) and prostaglandin E2 (PGE2), are produced by inflammatory cells after induction by BNP, which also modulates various cytokines (TNF-a, IL-12 and IL-10) and affects cell mobility[59]. Other mechanisms that can be implicated in the increase of the natriuretic peptides levels are the aggressive fluid administration and the reduced production of urine due to renal dysfunction which is very common in septic patients.

Finally many studies have been performed in order to discover a possible prognostic value of plasma BNP levels in septic patients. Some of these studies have concluded that high levels of BNP and NT-proBNP are connected with increased possibility of death in septic patients of the Intensive Care Unit[54,58,60]. Some investigators believe that the levels of these peptides can be used as diagnostic criterion of cardiac dysfunction in septic patients and can substitute invasive monitoring like the pulmonary artery cannulation[61-64], whereas other do not agree with these conclusions[65,66]. Lastly the level of BNP has been used as criterion for successful weaning of patients from mechanical ventilation[67].

Endothelins

Endothelin-1 is a peptide that was firstly discovered in 1988 and has a very powerful vasoconstrictive function[68]. It is mainly produced by endothelial cells but other cells have also been found to produce it like vascular smooth muscle cells, mucosa epithelial cells, macrophages, mast cells, myocardial cells, tracheal epithelial cells, medullary cells of the kidney, some nervous cells and Kupffer cells[69]. Endothelin is initially produced as pro-peptide which is cleaved to the final active substance by the action of various endopeptidases. The stimuli that trigger endothelin-1 production is mechanical stress of the endothelium (the main stimulus), hypoxia, endotoxins, TNF, IL-1, TGF, adrenaline, thrombin and angiotensin-2 while prostacyclin, NO, ANP and heparin inhibit the production of endothelin-1[69], Apart from endothelin-1 there are two other endothelins: endothelin-2, which is located mainly in the intestine, and endothelin-3 which is located in the lungs, central nervous system and the intestine too[70]. Endothelins activate their receptors which are endothelin receptor A (ETA), with greater affinity with endothelins 1 and 2, and endothelin receptor B (ETB) which is equally binded to all three endothelins[71].

In septic shock the plasma endothelin levels are significantly elevated[72] and their elevation seems to correlate with sepsis morbidity[73]. The great amounts of circulating endothelin are related to the pathophysiological disorders in various organs such as the heart, lungs, liver, intestine, kidney and others. Endothelin action on the heart involves constriction of coronary arteries, cardiac output reduction and arrythmogenesis, as it is proved in in vivo studies[69], and these actions are probably responsible for cardiac dysfunction during sepsis. Wanecek et al[74] concluded that antagonism of the myocardial endothelin receptors greatly improves cardiac function in septic models and that stimulation of these receptors by endothelin deteriorates the normal function of the myocardium. In the lungs, endothelin causes pulmonary hypertension, which is characteristic of sepsis-induced ARDS, by contracting the pulmonary arteries[75]. Other mechanisms of endothelin action on the lungs are generation of interstitial edema and leucocyte aggregation in the alveoli[64]. There are suggestions that endothelin overproduction significantly impairs intestinal epithelial function because endothelin receptor antagonism improves mucosal microcirculation of the intestine in various experimental models of sepsis[69]. Also it is related to hepatic dysfunction via constriction of the portal vein and the hepatic capillaries which cause hepatocellular destruction[69]. Finally the action of endothelin on the kidney and its relation to acute renal failure is not definitely proved. It is suggested that endothelin impairs renal blood flow and normal renal function[76,77] but these findings are not confirmed by all the investigators[78]. Important results are being waited by the study of effectiveness of endothelin antagonists in the treatment of septic shock.

Sepsis and septic shock were and remain important research topics for many scientists. Despite the progress in understanding the mechanisms of pathophysiologic

changes that these situations cause there are still questions that have not been answered. The most important is the transition from the hyperdynamic to the hypodynamic phase of sepsis and the lack of response of the peripheral vessels to exogenous vasopressors. Experimental findings indicate the important role of the endothelium and the endogenous vasoactive substances. A lot of studies have been performed in the pathophysiology of septic shock in order to use their knowledge for therapeutic purposes but very few of them have concluded in morbidity and mortality reduction[5]. Large multicenter studies are in progress and their results are being waited with high expectations.

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