Serotonin Signaling and the Hyperpermeable Endothelial Barrier in Sepsis: Clues to a Molecular Mechanism

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Short Summary
Sepsis is a serious medical condition characterized by a severe systemic inflammatory response caused by a microbial infection. This review evaluates a novel approach to therapy, which targets serotonin (5-HT) and its transporter, SERT.

Abstract
Sepsis is characterized by a severe systemic inflammatory response caused by hyperpermeability of the endothelial barrier resulting microvascular leakage, which is a leading factor to multorgan failure. In sepsis, the hyperpermeable endothelial cells contribute to the activation of platelets, which release numerous mediators that affect coagulation, inflammatory response and are believed to directly or indirectly affect the integrity of the endothelial barrier. One such mediator is serotonin (5-hydroxytryptamine, 5-HT), a signaling molecule which mediates a number of cellular functions including regulation of cytoskeletal dynamics associated with barrier function of endothelial cells. The actions of 5-HT are mediated by different types of receptors and terminated via an uptake mechanism of a 5-HT transporter (SERT) on the platelet and endothelial cell. Earlier studies revealed unexpected discoveries concerning the impact of 5-HT signaling on the permeability of the endothelial barrier. These findings have been supported by the clinical reports on the anti-inflammatory property of 5-HT reuptake inhibitor, SSRIs in treating sepsis-related morbidity and mortality. This review focuses on a wide-range of literature to pinpoint cellular and molecular mechanisms that mediate 5-HT-induced microvascular injury in sepsis pathogenesis.

Keywords: Sepsis; Platelets; Endothelial Barrier; Serotonin; Serotonin transporter; Serotonin signaling; SSRIs

Introduction
Sepsis is a medical condition characterized by a severe systemic inflammatory response caused by a microbial infection. In the U.S., sepsis causes ~270,000 deaths annually and a health care burden of ~$41.8 billion [1-3]. Sepsis is an important cause of morbidity and mortality in the older population (greater than 80 years) [2,3]. Patients with severe sepsis have a poor prognosis with mortality rates of 40%-60% when one or more organs are affected [2-4]. Yet, there are no effective therapies to treat sepsis [4-6], and clinicians rely only on supportive care [6-9] usually initiated after the presence of symptoms, and not necessarily improve the microcirculatory function [9].

Clinical studies reported the anti-inflammatory property of SSRIs, the serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors [10-14], in treating infectious disease-related morbidity and mortality [15-18]. The traditional view of the SSRIs invokes a functional stratification of serotonin transporter, SERT which is responsible for accumulation of 5-HT by neurons, platelets, and other cells. SSRIs reduces the reuptake rates of 5-HT into serotonergic neurons through acting on SERT and to increase the synaptic 5-HT levels. Because, the downregulation of 5-HT in synaptic cleft is the foundation of a variety of neuropsychiatric disorders, including affective disorder, anxiety disorders, obsessive-compulsive disorder, and autism [19-21]. Due to these characteristics, SSRIs have been used to treat the neuropsychiatric disorders [20].
5-HT can involve in numerous biological processes in the cell cytoplasm, and in extracellular compartments through receptor-dependent and receptor-independent (transporter, SERT-dependent) signaling pathways (Figure 1); for the stability of cellular events, extracellular vs cytoplasm ratio of 5-HT should be regulated [22-24].

Receptor-dependent pathway is related with the plasma 5-HT signaling, which is initiated by an interaction between plasma 5-HT and 5-HT receptors such as 5-HT2A, or 5-HT2B, on platelets or endothelial cells, respectively. 5-HT signaling transduced by 5-HT2A mobilizes calcium from intracellular stores to trigger the vesicular release of pro-coagulant molecules from granules [24-26]. SERT-dependent (receptor-independent) pathway is related the free level of 5-HT in cell cytoplasm which binds to small GTPases, such as Rab4 and regulates the membrane trafficking of granules as well as SERT [22,27-31]. Thus, SSRIs downregulate the cellular 5-HT uptake rates which elevates the plasma 5-HT concentration [29,30]. These studies emphasize the importance of plasma vs. platelet 5-HT ratio in platelet physiology [32].

As in neuropsychiatric disorders, the level of 5-HT becomes an important factor in development of various diseases. Once SSRIs are in the system, they target all the SERT proteins at neuronal and peripheral systems and SSRIs change the ratio of 5-HT in blood plasma vs cell cytoplasm. Therefore, any attempt to build a link between the anti-inflammatory role of SSRIs and sepsis must start with a detail analysis of cellular mechanisms to learn more about the mechanism if it is through elevating the extracellular 5-HT, or reducing the cytoplasmic 5-HT levels. Here, the reader is referred to a number of publications on detail background and additional perspective on 5-HT signaling pathway and the hyperpermeability of the endothelial barrier [33-39].

At elevated levels, 5-HT in blood plasma are associated with increases in G protein-coupled receptors signaling and serotonylation of small GTPases [25-28], which in turn lead to remodeling of cytoskeletal elements to enhance granule secretion and promote unique expression of sialylated N-glycan structures on smokers’ platelets [30-33]. In receptor-dependent pathway, 5-HT signaling elevates resting concentrations of intracellular Ca²⁺ and transglutaminase (TGase) activity which in turn accelerates the cellular trafficking dynamics and remodels the surface proteins and glycans [25-28]. MALDI/MS and LC/MS/MS analyses of the membrane proteins and glycans identified an elevation in the number of sialylated N-glycans [31], as well as the appearance of certain enzymes and proteins on the plasma membrane [32-34], included in membrane trafficking of secretory vesicles (via altering the actin-myosin network), that bind G-protein-coupled receptors, or that activate small GTPases [28]. These studies suggest a link between 5-HT signaling and its downstream effectors—including phospholipase C (PLC) and inositol-1,4,5-triphosphate (IP3) pathways, in receptor-dependent pathways (Figure 1). When the blood plasma 5-HT concentration is elevated, the 5-HT receptor on the endothelial cells, 5-HT2B sends the signal to activate PLC and produce IP3 (Figure 1). Rac-GTP is formed when TGase transamidates 5-HT. Binding to Rac-GTP activates PK1, which phosphorlylates vimentin. PK1 is a Rac1 effector and its activation leads to multiple phosphorylations that commonly occur at the plasma membrane [35].

Additionally, 5-HT-induced permeability of endothelial cells was associated with the phosphorylation of p21 activating kinase (PAK1), PAK-dependent phosphorylation of vimentin (P-vimentin) filaments [22,28,29,36,37]. Following phosphorylation, the curved filamentous structure of vimentin undergoes reorganization and straightens [22,28,29]. These findings need to be confirmed by further investigations with preclinical and in vitro study models, for example, if SSRI application restores internal organ damage in preclinical sepsis-induced mouse model. What is the mechanism that SSRI rescue the sepsis-associated endothelial hyperpermeability? Does SSRI act as an anti-inflammatory agent through elevating the extracellular 5-HT, or reducing the cytoplasmic 5-HT levels? Addressing these issues will advance our understanding of the mechanisms underlying cellular responses to refine current views of 5-HT signaling processes during sepsis.

While all in vitro models of sepsis have essential limitations, it is still necessary to investigate molecular mechanisms to develop effective therapies to treat sepsis. For these purposes, the preclinical models were developed to study sepsis mediate microvascular injury [38,47]. These include intravascular infusion of endotoxin or live bacteria, soft tissue infection, cecal ligation and puncture (CLP)-induced murine, rats or rabbit models. In lipopolysaccharide (LPS)-induced sepsis mouse model [43-47], Huang et al showed that the elevated 5-HT in blood plasma aggravated sepsis-induced acute lung injury by promoting neutrophil extracellular trap formation in the lungs of LPS-sepsis mouse [48]. LPS murine model uses younger mice which do not mimic the age of the human population that incurs sepsis [48,49]. In contrast, CLP murine model [43-47] uses older mice to more closely mimic the age of the human population that incurs sepsis, and the mice receive fluids and antibiotics to mimic the basic supportive therapy afforded patients with sepsis [50]. In between all other sepsis-induced animal models, CLP is generally accepted to be a relevant rodent model of bacterial sepsis because it exhibits many of the key pathogenic features observed in humans with severe sepsis during the hypotensive periods of cold shock [46]. Additionally, CLP-model is easy to monitor the steps in the development of sepsis such as the early failure of the...
renal microcirculation [45,47]. In CLP-sepsis mice, the decreased perfusion and leakage, which are a leading acute kidney injury and directly related to changes in organ function were observed by intravital microscopy [45,47]. Based on all these characteristics, CLP-induced sepsis mice model is accepted as a good model to learn more about sepsis.

In a recent study, Zhang, et al. used 5-HT deficient mouse model, TPH1-knockout (KO) mice in to develop sepsis [43]. TPH1 is the rate-limiting enzyme in the biosynthesis of 5-HT [25,51]. TPH1-KO mice do not have 5-HT in their blood system Zhang et al., the survival rates of CLP-induced wild-type with the CLP-induced TPH1-KO mice [43]. Interestingly, the CLP-induced wild-type mice had a significantly lower survival rate than the CLP-induced TPH1-KO group. The tissue histopathology analysis revealed that 5-HT markedly exacerbated histological damages in the peritoneum, lung, liver, kidney, intestinal tissue, and heart in sepsis. In this study, Zhang, et al. proposed that the initial elevation of plasma 5-HT promoted serum cytokines and bacteria as well as facilitating oxidative stress in sepsis [43]. It will be interesting to investigate if SSRI treatment will be able to recover the elevated 5-HT-associated damages.

There is a growing appreciation to the importance of microcirculatory failure in the development of organ injury during sepsis [52,53]. Indeed, microvascular dysfunction, increased microvascular permeability is a hallmark of sepsis and recognized now as a strong predictor of death among patients with severe sepsis [54,55]. We observed the declined renal microcirculation in mice few hours after CLP was induced [42]. Using CLP-induced mice model, we investigated the mechanism by which 5-HT regulates the microvascular permeability in development of sepsis [42]. Additionally, these studies addressed if clinically relevant delayed therapy with a SERT inhibitor restore microcirculatory perfusion and renal function [42]. When the CLP was induced on mice that lack the SERT gene (SERT-KO mouse [34]), the impact of CLP-sepsis on microvascular perfusion of these mice were much better than the only CLP-induced wild-type mice [42]. Also, mice treated with the SERT inhibitor, paroxetine, had better microvascular perfusion following CLP. Overall, reducing the 5-HT uptake rates via targeting SERT, either genetically or through SSRI could reduce sepsis induced microvascular leakage and help restore microvascular perfusion [42]. These findings appear to agree with the clinical reports on anti-inflammatory role of SSRIs in sepsis through reducing the cytoplasmic 5-HT levels.

The relationship between platelets and the endothelia is another case of the chicken-and-egg paradox. While the platelets are one of the major contributors to the endothelial damage [56-59], the hyperpermeable endothelial cells contribute to the activation of platelets [54]. There are several studies proposing the role of platelets in this major issue. Specifically, in vitro studies in the absence of tissue and endothelial wall injury demonstrated platelets responses to systemic immune complexes [56]. Based on these, platelets were proposed as a crucial mediator of the inflammatory response in sepsis [56]. Once it is activated, platelets release numerous mediators that affect coagulation, inflammatory response and are believed to directly or indirectly affect the integrity of the endothelial barrier [60]. One such mediator is 5-HT because platelets are the major storage for 5-HT in blood [39,61-63]. Therefore, in sepsis the initial elevation of plasma 5-HT is associated with the elevation of the serum cytokines, bacteria and oxidative stress. CLP-sepsis mice showed a significant elevation in 5-HT concentration in blood plasma [64,65]; furthermore, the 5-HT uptake rates of the renal endothelial cells exposed to septic serum or 5-HT showed an elevation in cellular 5-HT uptake rates [42].

In contrast to the relationship between the blood plasma 5-HT levels and the cellular 5-HT uptake rates in sepsis, our earlier studies reported that the cellular 5-HT uptake rates depended on the number of SERT molecules on the plasma membrane [22-24,27-31]. Moreover, an increase in extracellular 5-HT concentration reduced the cellular 5-HT uptake rates by decreasing the number of SERT molecules on the plasma membrane [22,29]. Like the other membrane proteins, SERT is translocated to the plasma membrane of the cells in a substrate-dependent manner [29]. Once 5-HT is removed from the extracellular matrix into the cytoplasm, SERT is rerouted from the plasma membrane to the cytoplasmic compartments. 5-HT signaling accomplishes this by altering membrane trafficking of small GTPases and the structure of cytoskeletal proteins [27]. Interestingly, in CLP-sepsis mouse, the relationship between the blood plasma 5-HT level and the cellular 5-HT uptake rates did not agree with these earlier reports [28]. The impact of the elevated serum cytokines, bacteria and oxidative stress on structure and function of SERT must be investigated to learn about the upregulation of 5-HT uptake rates in sepsis.

The impact of 5-HT on weakening the endothelial barrier was first described in the late 1950’s [61,62]. In this line, studies demonstrated that 5-HT increases the permeability of blood vessels [61,62], microvascular leakage [45,47,66] or recruitment of neutrophils [67]. Lately, a strong link was built between the anti-inflammatory property of the SSRIs with the concentration of 5-HT in extracellular compartment [10-15]. What is the mechanism by which septic conditions upregulate platelet and endothelial SERT and lead to loss of the endothelial barrier?

The findings from various laboratories propose that elevated 5-HT level activates PAK and disassembly and spatial reorientation of vimentin filaments [36,37,68-70] (Figure 1). Interestingly, these associations were found by an immunoprecipitation assay, only in
endothelial cells grown in CLP-mouse, not in SHAM-mouse serum [42]. These findings also confirm the impact of the level of 5-HT in extracellular compartment, which is elevated in CLP-sepsis serum. Vimentin is a type III intermediate filament involved in cytoplasmic trafficking in cells of mesenchymal (e.g., endothelium, fibroblasts, megakaryocytes) and myogenic origin [71,72]. Vimentin participates in a network of intermediate filaments that extends beneath the cell membrane and regulates the activity of PAK, which functions in the transduction of diverse extracellular signals to alter intracellular pathways [28,36,37]. Significantly, vimentin is a substrate of PAK, and PAK becomes activated in cells that are exposed to 5-HT. Following phosphorylation of the Serine at position 56, the curved filamentous structure of vimentin undergoes reorganization and straightens (37). As mentioned above, elevated 5-HT activates PAK to phosphorylate vimentin via changing the organization of this intermediate filament [22,29,36,37]. Following phosphorylation, the curved filamentous structure of vimentin undergoes reorganization and straightens. As a part of actin- myosin bundle, the structural change on P56-vimentin could also change the actin- myosin- vimentin cytoskeletal network [22,72,73]. Based on these data, we propose that during sepsis increased endothelial uptake of platelet-derived 5-HT together with 5-HT signaling leads to changes in the cytoskeletal network and its partner, endothelial (ve-cadherin), which in turn disrupts the endothelial barrier (Figures 1 & 2). Thus, agents targeting SERT could reduce sepsis induced microvascular leakage and help restore microvascular perfusion.

Figure 1: Proposed mechanisms by which 5-HT signaling and SERT-mediated increases in cytoplasmic 5-HT lead to weakening of the permeability barrier.

An association between P-vimentin and ve-cadherin involves in endothelial dysfunction during sepsis. This was deduced through our studies [42] where P-vimentin levels were highly increased in 5-HT pretreated platelets of wild-type mice however, SERT-KO mice could not phosphorylate vimentin even after 5-HT treatment. These findings suggest that phosphorylation of vimentin requires 5-HT uptake ability of SERT which upregulates the intracellular free 5-HT concentration [42] (Figure 2).

Figure 2: During sepsis, platelets release 5-HT which is taken up by endothelial SERT. Increased intracellular 5-HT along with extracellular 5-HT signaling lead to phosphorylation of vimentin and weakening of the tight junction. PAK1- dependent phosphorylation of vimentin produces a strong association between P-vimentin and ve-cadherin.

All these findings from clinical, preclinical, and in vitro studies build a strong link between blood plasma 5-HT level and sepsis-associated endothelial damage which suggest a facilitative role to the cytoplasmic 5-HT in sepsis-associated endothelial hyperpermeability. The following sections will summarize how SSRIs act an anti-inflammatory agent during sepsis. Addressing this gap is a crucial step in understanding of the existing body of knowledge in sepsis.
SSRI Protects Endothelial Barrier against Cytoplasmic 5-HT-Associated Damage

The endothelial membrane - cytoskeletal interactions play important role in the integrity of endothelial barrier function [71-75]. The interactions between complex sets of proteins that comprise tight junctions, adherens junctions, and gap junctions provide a barrier ability to the endothelial cells. Adherens junctions form pericellular zipperlike structures along the cell border through their transmembrane homophilic adhesion [73]. The adherens junctions in endothelial cells contain vascular endothelial (ve)-cadherin as the major structural protein that mediates homophilic binding and adhesion of adjacent cells in a Ca2+-dependent manner. ve-Cadherin is required for the proper assembly of adherens junctions and development of normal endothelial barrier function [71-75].

The endothelium of the vessel wall functions as a semi-permeable barrier between the blood and interstitial space and is important for tissue/fluid homeostasis. The loss of barrier function is a hallmark of sepsis and contributes significantly to overall microvascular failure [45,47,66]. Based on the literature and our findings [42], we propose that sepsis-associated elevation in 5-HT level in blood plasma leads to endothelial barrier disruption once it is taken in the cells. In cytoplasm, free 5-HT causes a structural reorganization of the vimentin, actin-myosin cytoskeleton leads an alteration in the conformation of cadherin, this weakens the endothelial cell barrier. However, if the uptake of 5-HT from extracellular compartments to the cytoplasm is hampered via SERT inhibitors like SSRIs, this rescues cytoplasmic 5-HT-associated damage on the endothelial barrier (Figure 2).

The endothelial cell lining of the vessel wall is the first line of defense against organ injury and functions as a semi-permeable barrier between the blood and interstitial space. As such, it is susceptible to injury from microbial virulence factors, proinflammatory mediators released from activated blood cells, and oxidative stress [44]. Briefly, microbial virulence factors, proinflammatory mediators in circulation, and oxidative stress promote the activation of endothelia in which the barrier function is impaired [54-56]. Endothelial hyperpermeability allows the neutrophil adhesion and infiltration into tissues [76], coagulation abnormalities [77], microvascular leakage [78], and hypoperfusion associated with sepsis-induced multiorgan failure [79] morbidity and mortality [80]; finally, endothelia cannot defense against organ injury anymore.

Based on the literature, it appears that the microbial virulence factors in circulation activates the endothelial cells which contributes to the activation of blood cells such as platelets. These white blood cells participate in the inflammatory response, hemostasis, host response, and microvascular permeability. Yet, a direct link between platelets and sepsis on an increased blood plasma 5-HT level has not been built until the clinical studies reported the advantageous of SSRIs in sepsis development [10-17]. Before this review, it was generally accepted that an environment favoring pathophysiological activation of platelets is related with the development of sepsis.

Platelets are derived from the fragmented cytoplasm of megakaryocytes and enter the circulatory system in an inactive form. In sepsis, the hyperpermeable endothelial cells contribute to the activation of platelets which accelerates the exocytosis of platelet cytoplasm located granules, dense and a-granules [9,10]. Notably, blood plasma 5-HT concentration is in the low nanomolar range, but the dense granules of resting platelets store millimolar concentrations of 5-HT [22-24,51,81]. Thus, platelets apparently are designed to tightly control the cytoplasmic free 5-HT concentration. However, secretion of dense granules from platelets increase the 5-HT concentration in the blood plasma several folds and in cytoplasm if SERT on platelet surface reuptake them [22-24]. In summary, there is a biphasic relationship between blood plasma 5-HT elevation, loss of surface SERT, and depletion of platelet 5-HT [51]. Specifically, in platelets, plasma membrane SERT levels and platelet 5-HT uptake initially rise as plasma 5-HT levels are increased, but then fall below normal as the plasma 5-HT level continues to rise. The actions of 5-HT are mediated by receptors, and terminated by a single 5-HT transporter, SERT, on the platelet and endothelial cell, through an uptake mechanism [28-34].

This Review summarizes the available literature on the anti-inflammatory roles of SSRIs in sepsis. Several evidences are provided demonstrating how sepsis-induced elevation in 5-HT levels in blood plasma, or in cell cytoplasm reorganize the intracellular factors to produce hyperpermeable endothelial barrier through engaging in receptor-dependent and -independent signaling pathways. Also, a crosstalk between the platelet and endothelia in sepsis was depicted. Based on existing evidences and emerging mechanistic insights, novel therapeutics targeting to stabilize the 5-HT level in endothelial cell cytoplasm in sepsis should be designed.

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