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REVIEW

The endocannabinoid system in sepsis – a potential target to improve microcirculation?

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ABSTRACT

During the last decade, research has identified the endocannabinoid system (ECS) as a key regulator of essential physiological functions, including the regulation of microvascular and immune function. Indeed, increasing evidence now suggests that release of endocannabinoids and activation of cannabinoid receptors occurs during sepsis and that manipulation of the ECS may represent an important therapeutic target to improve microcirculation in sepsis. In this review, the pharmacology and physiology of the ECS and the involvement of cannabinoids, cannabinoid receptors and non-CB1R/CB2R pathways related to ECS activation will be described. This information will increase our comprehension of the role of lipid signaling pathways in sepsis and may lead to the identification of new drug targets for the treatment of impaired microcirculation.

Key words: systemic inflammation, sepsis, microcirculation, lipid mediators, cannabinoids, cannabinoid receptors

Introduction

Sepsis, severe sepsis and septic shock are the most frequent causes of death in surgical intensive care unit patients (1) and generate significant costs to the health care system. (2) Of particular concern is the 75% increase in the incidence of sepsis that has been observed over the last 2 decades. Furthermore, the incidence is projected to continue to rise significantly as the population ages and the presence of co-morbidities also increases. (3,4) While there have been some advances in the understanding and treatment of sepsis, in many cases, despite timely intervention, these therapies are unable to abrogate sepsis pathophysiology. This suggests that the current therapeutic approaches and the underlying hypotheses guiding treatment for sepsis still remain inadequate.

'Cannabinoids from the plant, *Cannabis sativa*, have been widely used in medicine for over a millennium as anticonvulsant, analgesic, anti-emetic, anti-inflammatory and immunosuppressive drugs'. (5) The recent interest in cannabinoids was triggered by the discovery of endogenous cannabinoid receptors

and their ligands, anandamide and 2-arachidonoyl glycerol (2-AG). Cannabinoid-1 receptors (CB1R) exist primarily on central and peripheral neurons, their major role is to modulate neurotransmitter release, whereas the cannabinoid-2 receptors (CB2R) are found mainly on immune cells and are known to play a role in immune response and regulation of inflammatory processes. (6) The endocannabinoid system (ECS) is upregulated during local and systemic inflammation, e.g. sepsis. (7) However, the role of the ECS in the immune response is still not completely known.

In this review, we will briefly describe experimental models to study microcirculatory changes during sepsis and discuss experimental findings and potential clinical approaches to improve the impaired microcirculation in sepsis. Based on general aspects of lipid signaling in inflammation and sepsis, we will then discuss the pharmacology and physiology of the endocannabinoid system, involvement of cannabinoids and cannabinoid receptors and participation of non-CB1R/CB2R pathways related to ECS activation.

Experimental models to study the microcirculation in sepsis

Various experimental models have been described to study the microcirculation in sepsis. Currently, there are two main categories of sepsis models: 1) toxin models, e.g. endotoxemia and, 2) bacterial sepsis models, e.g. cecal ligation and puncture (CLP).

The toxin models represent acute septic models and are usually generated by direct administration of endotoxin intraperitoneally or intravenously. These toxin models are widely used to study the microcirculation in sepsis because of their favorable reproducibility and similarities to the pathophysiology of sepsis. (8) However, the disadvantage of these models is the massive and rapid production of inflammatory cytokines, which is not comparable to clinical patients with sepsis. The differences in cytokine response, most likely explains why therapeutic strategies that might be effective in toxin models are not effective in sepsis patients. (9)

Bacterial sepsis models are generated by introduction of bacteria into the body directly or indirectly. These models are chronic and more clinically relevant in comparison to toxin models. Bacterial sepsis can be generated by fecal pellets, defined bacterial inoculum or endogenous fecal contamination as described below. (10,11)

1) The fecal pellet models are induced by administration of feces plus an adjuvant (such as barium) within a pellet. The role of adjuvant in this fecal model is to prevent rapid clearance of bacteria by the host and to modulate the mortality rate. (12) The mortality rate in this model depends on dose and composition of the bacteria in the feces. The disadvantage of this model is the variation in the amount of bacteria and species between feces samples resulting in inconsistent sepsis severity. (13)

2) The defined bacterial inoculum models are very similar to the fecal pellet models. The only difference is that the bacterial population is defined in this model. Generally, *Escherichia coli* (*E.coli*) is the strain of bacteria that is mixed with sterilized cecal contents and given to the peritoneum. (14) The main advantage of this model is that the mortality rate is controllable by modifying the dose of *E. coli*.

3) Endogenous fecal contamination can be achieved by at least two models. In the CLP model the cecum is ligated and punctured with a needle. The advantage of this model is that the mortality rate can be modulated by the size of the needle used and the number of punctures made in the cecum. However, the disadvantages of this model are that animals are in general not fluid resuscitated and do not receive antibiotics. (15) A second type of fecal contamination is the Colon Ascendens Stent Peritonitis (CASP) model in which a stent with a defined diameter is placed into the ascending colon, allowing feces to reach the peritoneal cavity continuously. Compared to CLP the advantage of the CASP model is having a secured continuous fecal contamination and controllable mortality rate by modulating the size of the stent. (16)

Changes in the microcirculation during experimental and clinical sepsis

The main role of microcirculation is to provide oxygen and nutrients to the tissue cells. Impairment of microcirculation represents a key event in the pathophysiology of sepsis. (17) Using intravital videomicroscopy (IVM), a method that has been widely used to visualize the dynamical changes of microcirculation *in vivo*, researchers have demonstrated that alterations of the microcirculation, including a decrease in perfused capillary density and an increase in spatial heterogeneity of perfused capillaries, occurs in sepsis. (18) In a CASP-induced sepsis, microcirculation alteration started about 12 hours after insertion of a 16G stent, expressed by increased number of rolling and adhering leukocytes in small blood vessels, and reached significant differences at 18 h compared to control animals. However, there were no significant changes on the functional capillary density. (16) Using an endotoxemia model of sepsis we demonstrated that LPS administration decreases the rolling flow, increases the number of adhering leukocyte (more pronounced than in the CASP model of sepsis) and reduces functional capillary density in muscular and mucosa layers of the intestinal wall. (19) To compare the impact of different types of shock (septic vs. hemorrhagic) on microcirculation, Nencioni et al. demonstrated that the septic group of animals had more pronounced alterations in the microcirculation in comparison to the hemorrhagic group. (20)

The microcirculation in human sepsis can also be evaluated by video-microscopic techniques such as orthogonal polarization spectral (OPS) and sidestream dark-field (SDF). (17,19) Both techniques are based on the application of different wavelength lights to the tissue, with the reflection of light providing images from microvascular

vessels. (21) In a study by Chierego et al. (22) the OPS technique was used in 50 subjects (severe sepsis patients vs. healthy volunteers). The observations showed a significant decrease in the proportion of perfused vessels, from 90% to 48% in septic patients compared to control patients. (21,22)

Changes in microcirculation are commonly seen in critically ill patients, especially sepsis patients. Therefore, monitoring the changes in microcirculation could contribute to the diagnosis of sepsis and to the identification of therapeutic strategies to improve the outcome of microcirculatory dysfunction in septic patients.

Therapeutic approaches to improve the microcirculation in sepsis

According to the guidelines for the management of severe sepsis and septic shock, recommended therapies for the treatment of sepsis include: Initial resuscitation, antibiotic therapy, fluid therapy, vasopressors, corticosteroids, and recombinant human activated protein C. (23) However, no specific therapies are approved to treat the impaired microcirculation in sepsis. The lack of proven therapies is mainly due to the difficulties in measuring the impact of potential beneficial treatments on the microcirculation in humans.

Nevertheless, numerous approaches to improve the microcirculation in sepsis have been studied. The spectrum of agents tested includes: Vasoactive drugs (e.g. dopexamin), oxygen free radical scavengers (e.g. aminosteroids), anti-inflammatory drugs and anticoagulants. (20,24-26) Also, several antibiotics which possess vasoactive and anti-inflammatory properties may ameliorate decrements in microcirculation function. (27) Another approach is the use of different crystalloid and/or colloid fluids. (28,29) Last but not least, tight blood glucose level control has been shown to be beneficial for the septic microcirculation. (30)

Despite improvements of treatment with the described recommended approaches, microcirculatory therapies still remain unsatisfactory; new drug treatments aimed at identifying receptors and downstream signaling pathways during the first stages of severe sepsis is an area that is attracting attention for the development of newer agents that can be included in the drug treatment arsenal for sepsis patients.

Recently, considerable interest has been shown in the involvement and manipulation of endogenous lipid signaling pathways in sepsis progression and treatment. (31) Among candidate lipid signaling pathways, evidence suggests that the ECS may play a pivotal role in immunomodulation and microvascular regulation and therefore may be a therapeutic target in the treatment of sepsis.

General aspects of lipid signaling in inflammation and sepsis

Inflammatory reactions generally have a protective role in the body. In fact, acute inflammation is the response of the host to an infection or injury, and is orchestrated by neutrophil recruitment to the site of inflammation. (32) Uncontrolled inflammation is the cause of many diseases such as atherosclerosis, asthma, cancer and sepsis. (32) Inflammatory reactions must be resolved to prevent the spreading of acute inflammation to become chronic or cause disease. There is a growing body of evidence that inflammation and inflammatory disorders, including sepsis, could be modulated by endogenous chemical signaling molecules such as lipid mediators. The fundamental role of lipid mediators is regulating resolution of inflammation through activation of anti-inflammatory and pro-resolving signaling pathways. The arachidonate-derived eicosanoids, lipoxins, are the first recognized lipid mediators (LMs) that have the dual properties of anti-inflammation and pro-resolution. (32) Lipoxins reduce the entry of neutrophils to the site of inflammation, and increase the uptake and clearance of apoptotic neutrophils by macrophages. (33) The resolution function of lipoxins is mediated through activation of macrophages to initiate macrophage phagocytosis of the apoptotic neutrophil. (32) Another potential therapeutic application that lipid mediators such as lipoxins may have for the treatment of sepsis is their ability to block secretion of TNF by directly affecting human T-cells. (33) Given that blocking pro-inflammatory cytokine production is one potential approach for treatment of sepsis, identification of lipid signaling pathways that mediate anti-inflammatory actions is a key area of investigation.

In addition to lipoxins, the endocannabinoid system is upregulated during local and systemic inflammation such as sepsis. Endocannabinoids are released in response to inflammatory stimuli and their levels are elevated in the patients and animals with septic shock. Endocannabinoids are arachidonic acid derivatives and part of the bioactive lipid signaling system. Identification of the role of endocannabinoids in treatment of uncontrolled inflammatory diseases such as sepsis may reveal novel drug targets for this disease.

Endocannabinoid pharmacology and physiology

Research on the marijuana plant, *Cannabis sativa,* started in the 19th century. *Cannabis sativa,* which contains more than 60 active phytocannabinoids, is one of the oldest plants to be used for medical purposes including, as an analgesic, and in the treatment of obesity and cancers. Cannabinoids mediate their effects through binding to specific receptors, members of the G protein coupled receptor (GPCRs) superfamily. In 1990 Matsuda et al. (34) cloned the CB1R and in 1993, Munro et al. (35) identified the CB2R. CB1Rs are expressed in the central nervous system (CNS), especially in the brain, and have a role in regulation of synaptic transmission. CBR2 are mainly expressed on immune cells and activation of CB2R affects immune responses and inflammatory reactions. (36) Further investigation of CB2R expression showed that these receptors are not just restricted to the periphery but found

in select areas of the brain and in the gastrointestinal (GI) system. (37) The primary research focus of CB2Rs is their role in the immunological function of leukocytes. Accumulating experimental data has provided convincing evidence that modulation of the CB2R is a good target for treatment of inflammatory diseases. (38)

The ECS is an endogenous lipid signaling system. The ECS consists of cannabinoid receptors (CB1R and CB2R), endogenous ligands for these receptors (endocannabinoids), and the enzymes that are required for synthesis and inactivation of endocannabinoids. (36) Cannabinoids, the ligands for cannabinoid receptors, are divided into three main classes: Phytocannabinoids (cannabis derivatives), synthetic cannabinoids and endogenous cannabinoids (endocannabinoids). Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) are the most well known phytocannabinoids. (37) The *Cannabis* plant contains cannabinoids with varying pharmacological effects; THC is mainly associated with the behavioral effects of *Cannabis sativa*, but is also known to be analgesic and neuroprotective. (5) The phytocannabinoid, CBD, lacks psychoactive actions but is known to reduce inflammation, convulsion, anxiety and nausea. CBN is another component from the cannabis plant. It acts as a weak agonist of CB1R and CB2R and lacks the behavioral actions of THC. (37) Endocannabinoids are the body's natural agonists for cannabinoid receptors. In fact, endocannabinoids are members of the eicosanoid super family and are able to activate cannabinoid receptors. (39) The two most well known endocannabinoids are anandamide (AEA) (40) and 2-arachidonoyl glycerol (2-AG) (41) both of which function as neurotransmitters in the CNS and periphery. Anandamide binds to CB1Rs but can also bind to a lesser extent to CB2Rs. 2-AG binds with the same affinity to CB1R and CB2R and acts as a full agonist at both receptors. (5) Moreover, 2-AG and Anandamide are both synthesized "on demand" through multiple biosynthetic pathways. (42) In addition to endocannabinoids and plant phytocannabinoids, synthetic cannabinoids, derived through laboratory synthesis, are also available and exert their pharmacological actions by binding to either CB1R or CB2R, or both receptors. (37) Cannabinoid receptors activate multiple signaling pathways including preferential activation of G_i proteins, inhibition of adenyl cyclase (AC) and reduction in cAMP accumulation, and activation of mitogen activated protein kinase pathways in most tissues. (43) In the nervous system, CB1R activation results in inhibition of voltage-dependent calcium channels, as well as both activation and inhibition of voltage-dependent potassium channels.

Cannabinoids in inflammation and sepsis

The ECS plays an important role in immune system modulation and increasing evidence supports upregulation of the ECS during both local and systemic inflammation, e.g. sepsis. Endocannabinoids are released from macrophages, dendritic cells, platelets and parenchymal cells in response to inflammatory stimuli and oxidative stress, and are present in elevated levels in the sera of patients and animals in septic shock. (43,44)

Examination of CBR function has revealed that CB2R are present on macrophages, neutrophils and lymphocytes and activation of these receptors has been generally associated with anti-inflammatory effects including reduced macrophage and neutrophil numbers at the site of infection and decreases in pro-inflammatory cytokines. (45) The use of CB2R agonists in experimental models of moderate sepsis reduced the continued recruitment of neutrophils to the site of infection, while increasing phagocytosis and clearance of bacteria. (46) Conversely, in severe sepsis where neutrophil recruitment and function can be detrimental to the host through increased tissue damage and cardiac death early in the septic course, CB2R inactivation reduced mortality. (47)

With respect to the contribution of CB1R to inflammation and sepsis, several studies suggested that activation of CB1R located on the presynaptic terminals of autonomic nerves or the vascular walls may contribute to the hypotension associated with septic shock. (48) However, the contribution of CB1R in this action was called into question, when LPS still produced an acute hypotension response in mice genetically lacking both CB1R or CB1R/CB2R. (49)

Our group studied the effects of ECS modulation on the microcirculation in experimental endotoxemia and CASP-induced sepsis. We found that in endotoxemia, reciprocal activation of the ECS might be of benefit; stimulation of CB2R by HU308 reduced inflammation and improved microcirculatory parameters, inhibition of CB1R by AM281 was protective in the intestinal and the iridal microcirculation in experimental endotoxemia. (50,51) However, following 16 hours of CASP-induced experimental sepsis and treatment with the CB1R agonist, ACEA, leukocyte adhesion was reduced, whereas CB2R stimulation did not affect leukocyte adhesion. CB2R inhibition by AM630, however, significantly reduced leukocyte activation and restored capillary perfusion. (52)

Taken together, the evidence suggests that release of endocannabinoids and activation of cannabinoid receptors occurs during sepsis and that manipulation of the ECS may represent an important therapeutic target in managing sepsis and septic shock.

Non-CB1R/CB2R pathways involved in ECS activation

It has been recognized that endocannabinoids, phytocannabinoids and synthetic cannabinoids can activate receptors other than CB1R and CB2R. (45) Best known of the non-CB1R/CB2R cannabinoid-related receptors is the transient receptor potential vanilloid subfamily, member 1 (TRPV1). (53) This receptor is a ligand-gated cation channel that is a member of a superfamily of ion channels that also includes "classical" transient receptor

potential channels (TRPC), transient receptor potential melastatin channels (TRPM) and transient receptor potential ankyrin (TRPA) channels. (54) TRPV1 is found on sensory nerves and plays an important role in the transmission of pain. The endocannabinoid, AEA has been demonstrated to bind to this receptor, resulting in channel deactivation and analgesia. (55) In addition to TRPV1, TRPV2, TRPA1 and TRPM8 also respond to cannabinoid ligands. (45)

The orphan G protein coupled receptors, GPR55 and GPR18, are both activated by a variety of exogenous and endogenous cannabinoids and lipids, including the cannabidiol analogue, abnormal cannabidiol (abn-CBD), AEA and N-arachidonoyl glycine. Both GPR55 and GPR18 have been suggested as candidate receptors for the abn-CBD-sensitive cannabinoid-related receptor that mediates hypotension and alterations in macrophage activity. (56,57)

Conclusions

In summary, inflammatory diseases, including sepsis, are characterized by pathological changes within the microcirculation. (58) Long-lasting impairment of the microcirculation causes severe decrements in tissue perfusion and organ function and plays a key role in the progression to severe sepsis. (58) Given the complex pathophysiology of systemic inflammation and the high mortality associated with severe sepsis, identifying novel drugable targets and appropriate therapeutic windows is a priority for treating this disease.

During the last decade, research has identified the ECS as a key regulator of essential physiological functions, including the regulation of microvascular and immune function. Indeed, increasing evidence now suggests that release of endocannabinoids and activation of cannabinoid receptors occurs during sepsis and that manipulation of the ECS may represent an important therapeutic target in managing sepsis and septic shock. However, in order to move these findings into the clinic, it still remains essential to provide a more comprehensive understanding of ECS activity during sepsis. This will require further examination of: 1) ECS function in relevant experimental models of inflammation and sepsis using appropriate techniques, such as IVM. 2) Identification of target receptor proteins (both cannabinoid and non-cannabinoid receptors) involved in mediating the actions of cannabinoids and endocannabinoids in both immune cells and microvasculature. This information will increase our comprehension of the role of lipid signaling pathways in inflammation and may lead to the identification of new drug targets for treating sepsis.

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