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REVIEW

Targeting the Endocannabinoid System to Treat Sepsis

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ABSTRACT

The endocannabinoid system represents a potential therapeutic target in sepsis due to the presence of cannabinoid receptors (CB2) on immune cells. In this review we discuss how various targets within the endocannabinoid system can be manipulated to treat the immune consequences of sepsis. One of the targets outlined are the endocannabinoid receptors and modulation of their activity through pharmacological agonists and antagonists. Another therapeutic target covered in this review is the modulation of the endocannabinoid degradative enzyme's activity. Modulation of degradative enzyme activity can change the levels of endogenous cannabinoids thereby altering immune activity. Overall, activation of the CB2 receptors causes immunosuppression and can be beneficial during the hyperactivated immune state of sepsis, while suppression of the CB2 receptors may be beneficial during a hypoimmune septic state.

Key words: sepsis, endocannabinoid system, inflammation, immune modulation

Introduction

Sepsis is a complex immune disorder that can affect the function of almost all organ systems in the body. This disorder is characterised by a malfunctioning immune response to an infection that involves both pro-inflammatory and immunosuppressive mediators. This leads to severe damage and failure of vital organs, resulting in patient death (figures 1,2). Sepsis, septic shock, and systemic inflammatory response syndrome are the leading causes of mortality in surgical intensive care unit patients internationally. (1) Within the United States alone, the incidence of sepsis is around 750,000 annually, (2) resulting in more than 200,000 deaths. (3)

Older individuals with fragile immune systems are at a higher risk of developing sepsis after invasive surgical procedures. Concurrently, the proportion of our ageing population has been increasing steadily over the years with 8% of the population being over 60 years old in 1950, 11% in 2009, and predicted to be 22% in 2050. (4) As a result, the incidence of sepsis in North America is projected to rise along with the risk of patient mortality. (2) Unfortunately, current management of sepsis is limited to supportive care, and therapeutic intervention is mainly hindered by the complex pathophysiology and heterogeneous immuno-inflammatory nature of the disease. (5) Although supportive care is beneficial to septic patients, there are no specific therapeutic options available that target the immune system to eliminate septic related mortality. All of these facets have resulted in a significant cost to the heath care system. (2)

The current lack of viable therapeutic treatment options for sepsis underscores our insufficient understanding of this complex disease. The endocannabinoid system, a key regulator of essential physiological functions including

the immune system, has recently emerged as a potential therapeutic target for sepsis treatment. The endocannabinoid system acquires its name from the plant Cannabis Sativa, which has been used medically to treat a variety of ailments, as well as recreationally for centuries. Cannabis Sativa contains more than 60 active phytocannabinoids with the primary phytocannabinoid Δ9-tetrahydrocannabinol (THC), (6) activating both endogenous endocannabinoid receptors. (7)

The endocannabinoid system and sepsis

The endogenous endocannabinoid signalling system can be thought of as consisting of three main elements that mediate its signalling. The first element consists of the endocannabinoids or bioactive lipid signalling elements. The second element is the endocannabinoid receptors that bind endocannabinoids. Finally, the third element encompasses proteins and transporters that mediate biosynthesis, transport and degradation of endocannabinoids. (8-11)

To-date, two endocannabinoid receptors have been identified: cannabinoid type 1 (CB_1) and cannabinoid type 2 (CB_2) receptors. These receptors are members of the Class A Rhodopsin-like family of G-protein coupled receptors and, therefore, contain seven transmembrane domains. Both CB_1 and CB_2, preferentially couple to heterotrimeric $G_{i/o}$ proteins and adenyl cyclases, but may also couple to other classes of G proteins. (8,9). CB_1 receptors were the first cannabinoid receptor to be molecularly cloned. (12) CB_1 receptors are expressed ubiquitously in the body, but are most abundant in the central nervous system (CNS), where they modulate neurotransmitter release. (13) In keeping with this, the behavioural effects of cannabinoid ligands are associated with the CB_1 receptor in the CNS. CB_2 receptors were discovered shortly after the identification of CB_1 receptors, (14) and their expression is largely restricted to immune cells such as lymphocytes, macrophages, and neutrophils. (15) The expression of CB_2 receptors in immune cells in humans is as follows; B cells > Natural Killer Cell > Monocytes > Polymorphonuclear Neutrophils > CD8 Leukocytes > CD4 Leukocytes. (16)

Cells involved in the inflammatory response can be broadly distinguished into two categories: cells present in tissues, and cells that travel in the blood. The cells present in the tissues comprise of mast cells, endothelial cells and tissue macrophages, while the inflammatory cells within the blood stream are platelets and leukocytes. Leukocytes are further subdivided into polymorphonuclear (multi-lobed nuclei) cells and mononuclear cells. The polymorphonuclear cells are subdivided into neutrophils, basophils, and eosinophils, while the mononuclear cells are divided into monocytes and lymphocytes. (17) The inflammatory cells present in the tissue are activated and secrete inflammatory mediators when physically damaged or chemically stimulated, prompting localization of leukocytes to the site of damage. Cells of the immune system release cytokines that are protein mediators to either increase or decrease the inflammatory response. The cytokine sub-classification includes interleukins (ILs), chemokines, interferons, colony stimulating factors, tumour necrosis factors (TNFs), and growth factors. (17) TNF-α and IL-1 are the main pro-inflammatory cytokines associated with the LPS induced hyper-immune response of sepsis, while IL-10 is associated with the subsequent immune suppression of sepsis immunopathology. (18)

Since the discovery of the cannabinoid receptors, extensive research has been focused on elucidating their functions; (19) we now know that the endocannabinoid system is involved in an array of diverse functions like immune modulation, cancer, pain processing, anxiety, depression, and neuroprotection. (20) This diverse influence of the endocannabinoid system has made it an attractive potential drug target for the treatment of a wide array of human disease. Multiple pharmacological approaches have also been devised to manipulate the endocannabinoid system ranging from development of synthetic cannabinoid receptor ligands, to compounds that alter the biosynthesis or degradation of endogenous endocannabinoid ligands. (21,22) Due to the predominance of CB_2 receptor expression on immune cells, it is likely that they play a role in the function of the immune system as well as the inflammatory response. (23) Therefore modulation of these receptors and their ligands for therapeutic benefit will be the main focus of this review.

Two endogenous endocannabinoids have been most extensively studied. (24) They are: arachidonoylethanolamide (AEA; previously known as anandamide), (25) and 2-arachidonoylglycerol (2-AG) (figure 3). (26) Both AEA and 2-AG have the ability to activate both CB_1 and CB_2 receptors. AEA and 2-AG are released locally and have a short duration of action; the concentrations of these endocannabinoids are tightly regulated to avoid excessive stimulation. (10,22) Extracellular levels of these two endocannabinoids are strictly regulated by the system to avoid excess stimulation. The endocannabinoids are rapidly eliminated from the extracellular environment through cellular reuptake followed by enzyme hydrolysis. (10) Two main enzymes are responsible for the cytosolic degradation of the endocannabinoids. Fatty acid amide hydrolase (FAAH) is a membrane bound serine hydrolase enzyme that is responsible for the hydrolysis of AEA as well as a large number of endogenous fatty acid amides (figure 3). (27,28) Monoacylglycerol lipase (MAGL) is the primary enzyme responsible for the degradation of 2-AG (figure 3), (28) however FAAH and a few other enzymes also have the ability to degrade 2-AG.

Therapeutic Targets

Effective treatment of sepsis requires the proper identification and understanding of the patient's septic state. Current understanding now indicates that sepsis immunopathogenesis starts with an early hyper-inflammatory response followed by a later anti-inflammatory

response (figure 1). (28,29) Due to this biphasic pathology of sepsis, immunosuppression through the endocannabinoid system may not always be a beneficial therapy. The early hyperinflammatory response is characterised by an uncontrolled over activation of the innate immune response to pathogens. During this phase, an excessive amount of pro-inflammatory mediators are released from immune cells to mobilize a defensive response to the pathogens. Unfortunately, this exaggerated response causes damage to the host's tissue, leading to hypoperfusion and eventual organ failure. (28) During this phase of sepsis, a reduction in the release of pro-inflammatory cytokines and mediators will be helpful to minimize tissue damage. Modulation of the endocannabinoid system, particularly CB_2, (23) to dampen down the inflammatory response during this phase of sepsis may be a beneficial therapy. Alternatively, patients in later stages of sepsis have a suppressed immune system, a state initiated by their own immune system as a mechanism to counteract the preceding hyperimmune response. Unfortunately, tissue damage and organ failure can continue to occur in this phase from the invading pathogens left in the system. As a result during this phase, modulation of the endocannabinoid system to upregulate the inflammatory response may help to reduce mortality by eliminating invading pathogens.

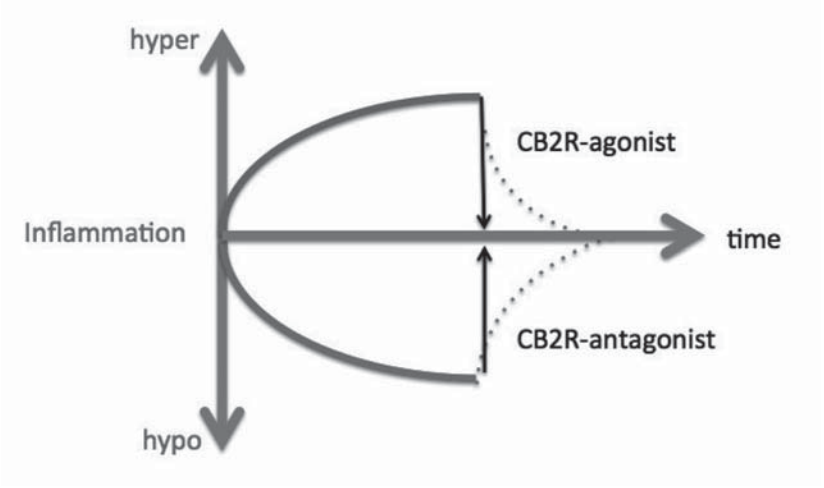

Figure 1. Immune response in sepsis and potential therapeutic endocannabinoid modulation.

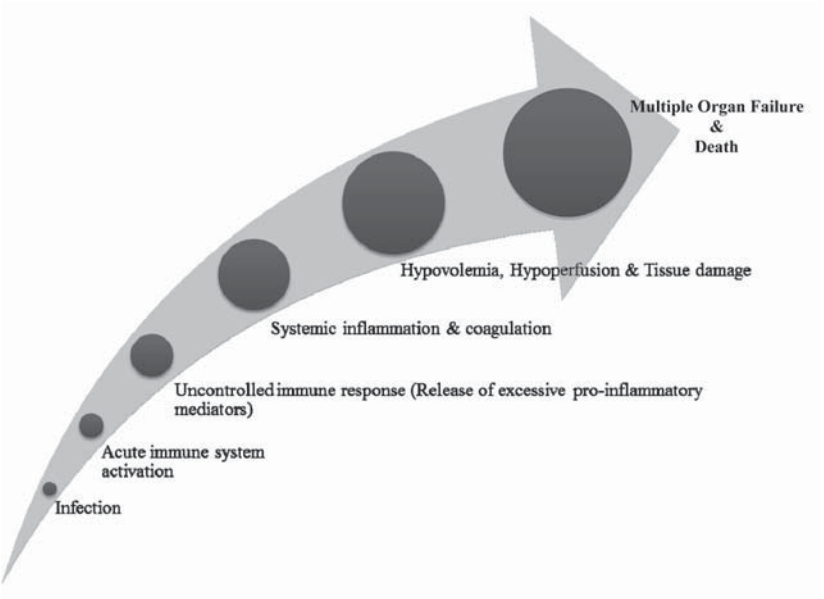

Figure 2. The sepsis cascade.

Endocannabinoid receptors

Modulation of the endocannabinoid system at the signalling elements, receptors or degradative enzymes, may provide a potential therapeutic benefit during inflammation and sepsis. One viable option is to directly target cannabinoid receptors using receptor selective drugs, where modulation of specific receptors can result in therapeutic benefit. (30) Previous studies have indicated that activation of presynaptic CB_1 receptors on autonomic nerves and vascular walls contribute to the hypotension associated with septic shock. (31) This result is not conclusive however because when CB_1 knockout mice were subject to acute endotoxemia, acute hypotension was still observed. (32) Studies in our own lab have found that inhibition of the CB_1 receptor, using the antagonist AM281, protected microcirculation of the gut and iris during acute endotoxemia. (33,34)

Manipulation of CB_2 receptors may have good utility in sepsis treatment given the localized expression of these receptors to macrophages, neutrophils and lymphocytes. Activation of CB_2 receptors is generally associated with an antinflammatory effect, with a resultant decrease in pro-inflammatory cytokines and a reduction in macrophage and neutrophil recruitment. (35) However, research on the modulation of these receptors has also provided some contradictory results. (36,37)

A study by Tschöp et al. modulated the CB_2 receptor in a systemic septic model induced through cecal ligation

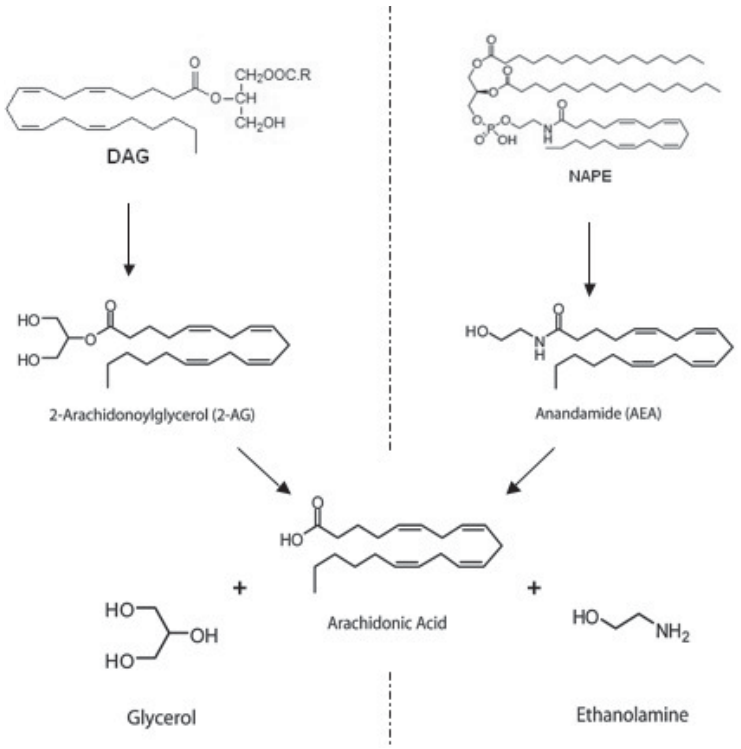

Figure 3. Production and degradation of endocannabinoids AEA & 2-AG. Phospholipid precursors are modified by N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) and Diacylglycerol lipase (DAGL) to produce AEA and 2-AG respectively. MAGL degrades 2-AG into arachidonic acid and glycerol, while FAAH degrades AEA into arachidonic acid and ethanolamine.

and puncture (CLP) in mice. (36) They observed reduced survival time of CB_2 receptor knockout mice compared to wild type mice during systemic sepsis. Furthermore, the CB_2 receptor knockout mice showed increased neutrophil recruitment and decreased neutrophil activation at the site of infection, leading to additional inflammatory damage. To observe CB_2 receptor involvement in inflammatory mediation, they also used a highly specific CB_2 receptor agonist called GP1a in wild type mice and observed increased mean survival time, decreased neutrophil recruitment, and increased neutrophil activation at the site of infection. Finally, the activity of a MAP kinase p38 known to regulate neutrophil function and priming was shown to be increased at the site of infection and associated with CB_2 activation. (36) Altogether, these results indicated that activation of the CB_2 receptor during systemic inflammation can help reduce mortality and tissue damage.

Another study by Csoka et al. also used a systemic inflammation model of sepsis through cecal ligation and puncture. (37) However, in the findings of this study they showed that CB_2 receptor knockout mice have significantly lower mortality rates as well as decreased number of bacteria within the blood, but similar levels of bacteria in the peritoneal lavage fluid when compared to wild type mice. They also measured serum levels of IL-10, an anti-inflammatory cytokine produce by macrophages, because an overproduction of this cytokine can impair immune defense to bacterial pathogens. They observed lower levels of IL-10 in both the blood serum and peritoneal lavage of knockout mice compared to wild type. All of these results indicated that CB_2 receptor activation during systemic sepsis increased IL-10 overproduction causing an increase in bacterial invasion into the blood, ultimately resulting in increased mortality rates. (37)

Multiple factors may help explain the inconsistency reported in studies of manipulating the CB_2 receptor during a septic state. (36,37) A possible explanation could be the severity of sepsis, where certain factors may play a crucial role in determining the role of the endocannabinoid receptors. Studies from our own lab indicate that in a more chronic stage of sepsis, inhibition of CB_2 receptors reduced leukocyte activation and restored capillary perfusion. (34) Furthermore the stage of sepsis immunopathogenesis could be different between the mice used in these two studies. (36,37) For example, activation of the CB_2 receptors during the immunosuppressed stage of sepsis can cause increased mortality, possibly explaining the results seen by Csoka and colleagues. Taken together, all these results indicate that early in disease progression of sepsis, activation of the CB_2 receptors may be beneficial given that it increases activation of neutrophils at the site of infection, while preventing additional neutrophil recruitment. However, in the latter stages of sepsis, an inhibition of CB_2 receptors may be beneficial in order to prevent additional inflammatory damage incurred by preventing the activation of more leukocytes. Additional research needs to be conducted in order to strengthen these conclusions.

Degradative Enzymes

Apart from manipulating the receptors themselves, regulation of endocannabinoid levels by targeting their enzymatic degradation may provide potential therapeutic benefit during sepsis. Although significant work has been carried out to-date on some of these endocannabinoid degradative enzymes, (10,27,38,39) since their discovery, to our knowledge, only a few studies have investigated the role of specific enzymes in sepsis. (40,41) With respect to FAAH, the key hydrolase for AEA degradation, cytokines such as IL-4 & IL-10 have been shown to influence FAAH activity

by increasing its activity, (40) and septic patients show a down-regulation of FAAH expression, leading to increased available AEA levels. (40,41) This is consistent with the elevation in AEA levels seen in experimental models in which FAAH activity is lost. (42) Increased AEA levels may be beneficial during the initial stages of sepsis to stimulate CB_2 receptors and help combat the exaggerated immune response. However, sustained elevated AEA levels can also be detrimental in the latter stages of sepsis by further suppressing the immune system, causing a heightened vulnerability to the invading pathogens. To our knowledge, the role of MAGL inhibition on sepsis and inflammation has not yet been documented, but chronic loss of MAGL function is associated with increased 2-AG levels, CB_1 desensitization and hyperalgesia. (43)

Our lab has explored the therapeutic potential of FAAH inhibition in experimental sepsis (endotoxemia). Rats were treated with a selective FAAH inhibitor, URB597, at different dosages, prior to the induction of sepsis. Furthermore, a separate group of rats were also treated with a selective CB_2 antagonist along with URB597 and LPS, to verify the role of CB_2 receptor activation. Our results showed that an LPS challenge significantly increased leukocyte adhesion to the endothelial lining in the collecting and post-capillary venules of the intestinal submucosa. Furthermore, capillary perfusion, measured through functional capillary density (FCD), was significantly decreased in both the circular and longitudinal muscle layer of the gut, as well as the intestinal mucosal villi during the LPS challenge. The group pretreated with URB597 showed a prevention of the increased leukocyte adhesion promoted by LPS. URB597 pre-treatment also prevented the LPS induced reduction in FCD, however the effect was only seen at the higher dose of URB597 and only in the circular muscle layer of the gut. The use of the CB_2 receptor antagonist, AM630, along with LPS and URB597 reversed the actions of the FAAH inhibitor on leukocyte adhesion for both collecting and post-capillary venules. This result indicates that the protective effects of URB597 were mediated by CB_2 activation. However, AM630 administration did not have any significant impact on the effects of URB597 on the FCD either in the mucosa or intestinal muscle layers. (44)

Conclusion

The endocannabinoid system modulates the immune response in experimental sepsis. Manipulating the endocannabinoid system may have potential therapeutic benefit in clinical sepsis where immune and inflammatory dysfunction can be detrimental. Multiple targets exist within the endocannabinoid system, e.g. the system can be targeted at the level of receptors by administration of synthetic compounds, similar to the endocannabinoids, which either increase or inhibit receptor activation to provide the desired therapeutic effect. Alternatively, the endogenous enzymes that degrade endocannabinoids or cannabinoid-like lipids can also be targeted in order to manipulate the levels of endocannabinoids. Proper identification of the septic stage is crucial to determine the adequate therapeutic response that will be most beneficial. Due to the biphasic nature of sepsis immunopathology, immune suppression through endocannabinoid modulation can help mitigate the hyperimmune response during the early septic state, while immune activation may be beneficial in later stages.

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