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MESTRADO INTEGRADO EM MEDICINA

A SYSTEMATIC APPROACH TO THE ROLE OF THE ENDOCANNABINOID SYSTEM IN THE MICROVASCULAR DYSFUNCTION OF SEPSIS

FRANCISCO MANUEL MARTINS DOS SANTOS VILA REAL ALMEIDA

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DESIGNAÇÃO DA ÁREA DO PROJECTO

Biomedicina - Farmacologia e Terapêutica

TÍTULO DISSERTAÇÃO/~~MONOGRAFIA~~ (riscar o que não interessa)

A systematic approach to the role of the endocannabinoid system in the microvascular dysfunction of retinis

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Eu, Francisco Manuel Martins da Costa Vila Real Alves da, abaixo assinado, nº mecanográfico 201905580, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro que:

☒ Não procedi à utilização de ferramentas de *chatbox* generativo baseadas em *large language models* para nenhuma das tarefas no contexto do meu trabalho de Dissertação ou Monografia

☐ Procedi à utilização de ferramentas de *chatbox* generativo baseadas em *large language models* no contexto do meu trabalho de Dissertação ou Monografia, encontrando-se todas as interacções (*prompts* e respostas) transcritas em anexo bem como a indicação das aplicações utilizadas.

Neste sentido, confirmo que a eventual utilização de ferramentas de *chatbox* generativo baseadas em *large language models* no contexto do meu trabalho de Dissertação ou Monografia foi exclusivamente descrita na sequência de *prompts* e respostas transcritos em anexo e nas aplicações indicadas.

Faculdade de Medicina da Universidade do Porto, 10/03/2025

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Francisco Vila Real

Agradecimentos

Este trabalho representa os últimos 6 anos, cheios de lágrimas e frustrações, mas também cheios de alegrias e razões para se ser grato.

Agradeço aos meus pais e irmãos, pelo apoio constante e por suportarem as épocas de exame, as negativas e muito mais. Este exemplo permitiu-me levar a vida com a leveza que me (creio) caracteriza.

Aos meus avós, pelo exemplo das suas vidas de trabalho e pelo carinho e tempo que me dedicaram, que me moldou e molda em tudo o que sou e faço.

À Inês, que me acompanhou no bom e no mau, sempre como um exemplo de resiliência e (mitológica) alegria no trabalho, características que gostava de aprender e levar comigo o resto da minha vida.

Aos meus amigos, aos que fiz de novo e aos que mantive, que me permitiram viver uma vida mais rica, dentro e fora das portas do hospital.

À FMUP, por ter sido uma verdadeira escola de valores, onde aprendi como e o que quero ser quando for "grande".

Dirijo, também, um especial agradecimento à minha orientadora, Professora Doutora Maria Augusta, e ao meu co-orientador, Dr. Emanuel Matias, que, pela sua postura e paciência, me deram um exemplo de rigor e dedicação que gostaria de repetir durante a minha carreira.

Por fim, agradeço a Deus, por tanto tanto bem recebido e pelas ajudas de última hora.

Que me permaneça fiel e dedicado ao que vivi nestes 6 anos.

Trabalho de acordo com as normas da revista "European Journal of Clinical Pharmacology"

A systematic approach to the role of the endocannabinoid system in the microvascular dysfunction of sepsis

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Resumo

Uma abordagem sistemática ao papel do sistema endocanabinoide na disfunção microvascular da sépsis

Introdução: O sistema endocanabinóide desempenha um papel chave na regulação da inflamação, da resposta imune e na homeostasia vascular. Nos últimos anos, tem sido amplamente estudado como um alvo terapêutico promissor para diversas patologias, nomeadamente inflamatórias e autoimunes. A sépsis é uma síndrome clínica potencialmente fatal que resulta da desregulação imunitária e de uma resposta inflamatória exacerbada à infeção, induzindo uma disfunção endotelial generalizada e alterações microvasculares que conduzem à falência multiorgânica. No entanto, a interação entre a disfunção microvascular em contexto de sépsis e o sistema endocanabinóide ainda é pouco explorada na literatura científica.

Objetivo e métodos: Foi realizada uma revisão sistemática para analisar a relação entre o sistema endocanabinóide e a disfunção microvascular na sépsis. A pesquisa bibliográfica foi conduzida nas bases de dados PubMed, Scopus e Web of Science, tendo sido selecionados estudos que recorreram a modelos experimentais de sépsis nos quais a intervenção envolveu a modulação do sistema endocanabinoide/canabinoide e a função microvascular e/ou plaquetária foi avaliada. O risco de viés e a qualidade metodológica dos estudos foram avaliados com base numa escala padronizada desenvolvida por Cosme D. et al..

Resultados: Foram incluídos 11 estudos, dos quais 9 realizados *in vivo* em modelos animais e 2 *in vitro* em células humanas. O alvo molecular mais investigado foi o recetor CB2 (9 estudos), cuja ativação demonstrou estar consistentemente associada a uma redução da adesão leucocitária, sugerindo um efeito protetor contra a inflamação microvascular. Além disso, observou-se uma diminuição na produção de VEGF e efeitos antiangiogénicos com a ativação dos recetores CB1 e CB2. O recetor CB1, por sua vez, mostrou influenciar a resposta contrátil dos vasos de forma dependente do contexto experimental.

Conclusão: A modulação do sistema endocanabinoide apresenta potencial terapêutico para a sépsis, especialmente na prevenção de complicações microvasculares associadas à doença e na modulação de pressão arterial. Assim, a avaliação deste potencial em humanos é essencial para validar a relevância translacional destes achados e a sua aplicabilidade clínica.

Palavras-chave: endocanabinoide, CB1, CB2, TRPV1, sépsis, disfunção endotelial, microvascular, resposta contrátil vascular, disfunção vascular, adesão leucocitária, VEGF

Abstract

A Systematic Approach to the Role of the Endocannabinoid System in the Microvascular Dysfunction of Sepsis

Introduction: The endocannabinoid system plays a key role in regulating inflammation, immune responses, and vascular homeostasis. In recent years, it has been extensively studied as a promising therapeutic target for a variety of pathological conditions, including inflammatory and autoimmune diseases. Sepsis is a life-threatening condition driven by immune dysregulation and exaggerated inflammatory response to infection, leading to widespread endothelial dysfunction and microvascular impairment, that drive multi-organ failure. However, the interaction between microvascular dysfunction in sepsis and the endocannabinoid system remains poorly explored in scientific literature.

Objective and Methods: A systematic review was conducted to analyze the relationship between the endocannabinoid system and microvascular dysfunction in sepsis. A literature search was performed using PubMed, Scopus, and Web of Science databases, selecting studies that employed experimental models of sepsis in which interventions targeted endocannabinoid/cannabinoid modulation and evaluated microvascular and/or platelet function. The risk of bias and methodological quality of the studies were evaluated using a standardized scale developed by Cosme D. et al..

Results: A total of 11 studies were included, 9 of which were *in vivo* studies in animal models and 2 *in vitro* studies in human cells. The most frequently investigated molecular target was the CB2 receptor (9 studies), whose activation was consistently associated with a reduced leukocyte adhesion, suggesting a protective effect against microvascular inflammation. Furthermore, a decrease in VEGF production and anti-angiogenic effects were observed with the activation of CB1 and CB2 receptors. The CB1 receptor, in turn, was found to influence vascular contractile responses in a context-dependent manner.

Conclusion: Endocannabinoid system modulation emerges as a promising therapeutic approach for sepsis, particularly in preventing microvascular complications associated with the disease and modulating blood pressure. Therefore, evaluating this potential in humans is essential to validate the translational relevance of these findings and their clinical applicability.

Keywords: endocannabinoid, CB1, CB2, TRPV1, sepsis, endothelial dysfunction, microvascular, contractile response, VEGF, vascular function, leukocyte adhesion

Introduction

Cannabis sativa has been cultivated for over 4,000 years, playing a significant role in human civilization. Historically, it has been valued for its textile and construction applications, as well as for its psychoactive properties attributed to tetrahydrocannabinol (THC). Its medicinal use has long been recognized, with evidence suggesting anti-inflammatory, antidepressant and analgesic effects.[1].

The pivotal breakthrough in cannabinoid research occurred in 1990 with the discovery of the cannabinoid receptor type 1 (CB1), which provided new insights into the pharmacological potential of cannabinoids. This finding sparked the exploration of the endocannabinoid system (ECS), a complex network of receptors, endogenous cannabinoids, and enzymes responsible for the synthesis, degradation and transport of lipid-like molecules that regulate various physiological processes. The broader spectrum of proteins, enzymes, and lipids that influence ECS activity is collectively referred to as the "endocannabinoidome" [2].

CB1 and cannabinoid receptor type 2 (CB2) are the most studied and well-portrayed cannabinoid receptors. They belong to the family of G protein-coupled receptors (GPCRs), which inhibit adenyl cyclases and recruit arrestin-dependent pathways. CB1 receptors are particularly enriched in the nervous system but are found in peripheral tissues, including the liver, adipose tissue, and skin. The activation of central CB1 receptors is responsible for the psychotropic effects of cannabinoids [4]. In contrast, CB2 receptors are mostly found in immune tissues and cells and mediate immune responses, inflammation, neuropathic pain and bone remodelling, for example [5,6]. Addicionally, CB2 receptors have been detected in microglia and in neurons, during inflammatory states. [7]

Recent studies have highlighted the role of ECS in vascular function, particularly in blood pressure regulation and endothelial activity. The Malinowska review [8] described a triphasic vascular response to cannabinoids, with an initial bradycardia and hypotension (phase 1), a short vasopressor effect (phase 2) and, finally, prolonged vasodilation (phase 3). However, inconsistencies remain in the understanding of this response, with reports of WIN55212-2 and CP55940 (CB1 agonists) inability to induce the phase 2 vasopressor effect and a study [9] suggesting a possible involvement of transient receptor potential vanilloid 1 (TRPV1) in the effect's physiology. Moreover, the vasopressor effect has rarely been described in reports [10] and is not fully understood [11]. Endothelial activation is also correlated with cannabinoid modulation, effects on leukocyte adhesion, endothelial inflammatory responses and even in the pathogenesis of atherosclerosis, where endothelial dysfunction plays a pivotal role [12,13].

Sepsis consists in the life-threatening organ dysfunction caused by a dysregulated host response to infection and is associated with in-hospital mortality greater than 10% [14]. Its pathophysiology involves immune dysfunction and imbalanced inflammatory activity, resulting in endothelial activation, microvascular damage, and ultimately, multiple organ dysfunction syndrome (MODS). Endothelial integrity is compromised through a combination of other mechanisms during sepsis, such as platelet dysfunction, coagulopathy, oxidative stress, and glycocalyx degradation, all of which contribute to microcirculation damage and disease progression. Despite promising evidence of restoration of endothelial function [15], there are no treatment options available for this dysfunction. This also applies to coagulation dysfunction, which closely relates to the severity and progression of sepsis and closely related to and correlated with endothelial dysfunction [16]. Platelets have been shown to participate in the pathological process of sepsis, namely in

organ damage [17], through endothelial loss of integrity and through Toll-like receptors, where bacterial lipopolysaccharides (LPS) could stimulate platelet secretion and potentiate platelet aggregation, via TLR4 [18].

Given the ECS's role in maintaining vascular integrity and the critical involvement of endothelial dysfunction in the transition of sepsis to septic shock, cannabinoid-mediated modulation of endothelial responses during warrants further exploration. However, the precise mechanisms by which CB1 and CB2 receptor activation influence vascular responses in sepsis remain poorly understood. This review aims to synthesize the current evidence on the link between the endocannabinoid system and vascular dysfunction in sepsis, highlighting its potential as a therapeutic target for minimizing sepsis-related complications.

Methods

This systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A meta-analysis was not performed. We evaluated studies investigating the effects of cannabinoids on vascular homeostasis and hemostasis. The target population included experimental models of sepsis that incorporated an infectious component or an infection-based systemic inflammatory response (LPS). Eligible models included live animal models, human models, and *in vitro* studies. The intervention of interest was any modulation of the endocannabinoid system (e.g., antagonism, agonism, enzyme activity variations) within the context of sepsis. No restrictions were applied to comparators. The primary outcomes were endothelial integrity, platelet function and overall vascular competence. We included all parameters measuring these functions as reported in the selected studies. A secondary outcome was the potential therapeutic application of endocannabinoid ligands for sepsis and septic shock, particularly targeting platelet and endothelial dysfunction.

Eligible study designs included randomized controlled trials, animal model experiments, and in vitro studies. No restrictions were placed on language or publication year. A comprehensive literature search was performed in PubMed (1997–present), Web of Science (1999–present), and SCOPUS (1980–present), between October 28 and November 1, 2024, with the final search conducted on November 2, 2024. The following search query was applied across all platforms: [[cannabinoids] OR [endocannabinoids] OR [cannabinoid receptor]] AND [[inflammation] OR [systemic inflammatory response syndrome] OR [sepsis]] AND [[microvessels] OR [endothelium] OR [blood platelets] OR [hemostasis]] [All Fields].

Study selection occurred in two phases, conducted independently by two reviewers. Initially, titles and abstracts were screened for relevance. In the second phase, full-text articles were reviewed to determine eligibility. Data extraction was performed manually and independently by both reviewers, with disagreements resolved by discussion and consensus. Extracted data included: study authorship, title, publication year, intervention details, study population or model, methodology, cannabinoid receptor of interest, and main findings.

To assess methodological quality, we applied a 14-item quality checklist developed by Cosme D. et al. Each criterion was scored from 0 to 2 (0 = not reported, 1 = limited information, 2 = complete detail). The total score for each study was determined by summing individual criterion scores and normalizing against the maximum possible score (28). Quality assessment was conducted independently by two reviewers and is available in Table 1

.

Table 1: Quality assessment of the included genetic studies; scored as 0points (information not available in the paper); 1 point (limited information provided); 2 points (complete information regarding that aspect).

Study	Authors (Year)	Criteria														Overall score (median)	Maximum Score (%)
		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.		
Activation of Cannabinoid Receptor 2 Attenuates Leukocyte-Endothelial Cell Interactions And Blood-Brain Barrier Dysfunction under Inflammatory Conditions	Ramirez, Hasko, Skuba (2012)	2	1	2	2	2	2	1	0	2	2	2	2	2	1	1,64	82,14
LPS-mediated neutrophil VEGF-A release is modulated by cannabinoid receptor activation	Braile, Cristinziano, Marcella (2020)	2	2	2	2	2	2	2	0	2	2	2	2	2	1	1,79	89,29
Inhibition of endocannabinoid degradation in experimental endotoxemia reduces leukocyte adhesion and improves capilary perfusion in the gut	Kianian, Al-Banna, Kelly (2013	2	2	2	2	2	2	2	1	2	2	1	2	1	2	1,79	89,29
Cannabinoid 2 Receptor activation reduces leukocyte adhesion and improves capillary perfusion in the iridial microvasculature during Systemic inflammation	Toguri, Moxsom, Szczesniak 2015)	2	2	2	2	2	2	1	2	1	2	2	1	2	2	1,79	89,29
The Endocannabinoid/Endovanilloid N-Arachidonoyl Dopamine (NADA) and Synthetic Cannabinoid WIN55,212-2 Abate the Inflammatory Activation of Human Endothelial Cells	Wilhelmsen, Khakpour, Tran (2014)	2	2	2	2	-	2	2	0	2	2	2	2	2	1	1,77	82,14
Selective Activation of Cannabinnoid Receptor 2 in Leukocytes Supresses Their Engagement of the Brain Endothelium and Protects the Blood-Brain Barrier	Rom, Zuluaga-Ramirez, Dykstra (2013)	2	2	2	2	1	2	2	0	2	2	2	2	2	2	1,79	89,29
Potenciation of Anandamide Effects in Mesenteric Beds Isolated from Endotoxemic Rats	Orliac, Peroni, Celuch (2003)	2	1	2	2	2	2	2	0	2	2	2	2	1	1	1,64	82,14
Delta-9-Tetrahydrocannabinol conserves Cardiovascular Functions in a rat model of endotoxemia: Involvement of endothelial molecular mechanisms and oxidative-nitrative stress	Bányai, Répás, Miklós (2023)	2	2	2	2	2	2	1	0	2	2	2	1	2	2	1,71	85,71
Endocannabinoid-mediated modulation of Gq protein-coupled receptor mediates vascular hyporeactivity to nor-adrenaline during polymicrobial sepsis	Singh, Sharma, Nakade (2018)	2	2	1	2	2	2	2	1	2	2	2	2	2	2	1,86	92,86
CB2-receptor stimulation attenuates TNF-α-induced human endothelial cell activation, transendothelial migration of monocytes and monocyte-endothelial adhesion	Rajesh, Mukhopadhyay, Bátkai (2007)	1	1	2	2	2	2	2	0	2	2	1	2	2	1	1,57	78,57
Cannabinoid CB1 Receptor Antagonist Rimonabant Decreases Levels of Markers of Organ Dysfunction and Alters Vascular Reactivity in Aortic Vessels in Late Sepsis in Rats	Leite-Avalca, Staats, Verona (2019)	2	2	2	2	2	2	2	0	2	2	2	2	2	2	1,86	92,86
Average score (for each criterium)		1,91	172	1,91	2	1,73	2	1,73	0,36	1,91	2	1,82	1,82	1,82	1,55	1,75	86,69

Legend: 1.: Scientific background and explanation of rationale Scientific background and explanation of rationale; 2.: Definition of the specific objectives or hypothesis; 3.: Definition of endpoints to study; 4.:Accurate description of the laboratory methodologies (easy to understand ad described in enough detail to allow replication), definition of test compounds, experimental conditions, and other important information; use of validated methods; 5.: Ethical review permissions, when applicable; 6.: Descripton of the statistical methods, when adequate; 7.: Obtain valid data and ensure that it is reliable; 8.: Evaluation by independent observers; blinding; evidence of independent repetitions, 9.: Cite relevant scientific papers when presenting data; 10.: Accessible and transparent presantation of data throughout the paper (including the appropriate measures of precision/variance); 11.: Critical discussion of the results; comparison with relevant research on the field; 12.: Draw consistent conclusions based on the evidence presented in the paper; 13.: State the contribuition to cumulative scientific knowledge and the pratical implications of the finding; 14.: Disclose conflicts of interest and declaring funding sources

Results

The search retrieved 276 articles, 59 from Web of Science, 164 from Scopus, and 53 from PubMed. After removing 77 duplicates, 199 unique articles remained. Screening based on titles and abstracts led to the exclusion of 182 articles that did not meet inclusion criteria. Seventeen full-text articles were assessed for eligibility, of which six were excluded for lacking an experimental model of sepsis or endotoxemia. Ultimately, 11 studies met all inclusion criteria and were included in the final review. The study selection process is detailed in a PRISMA flow diagram (Figure 1). Key study characteristics and findings are summarized in Table 2 and Table 3, regarding results of animal studies and *in vitro* studies, respectively.

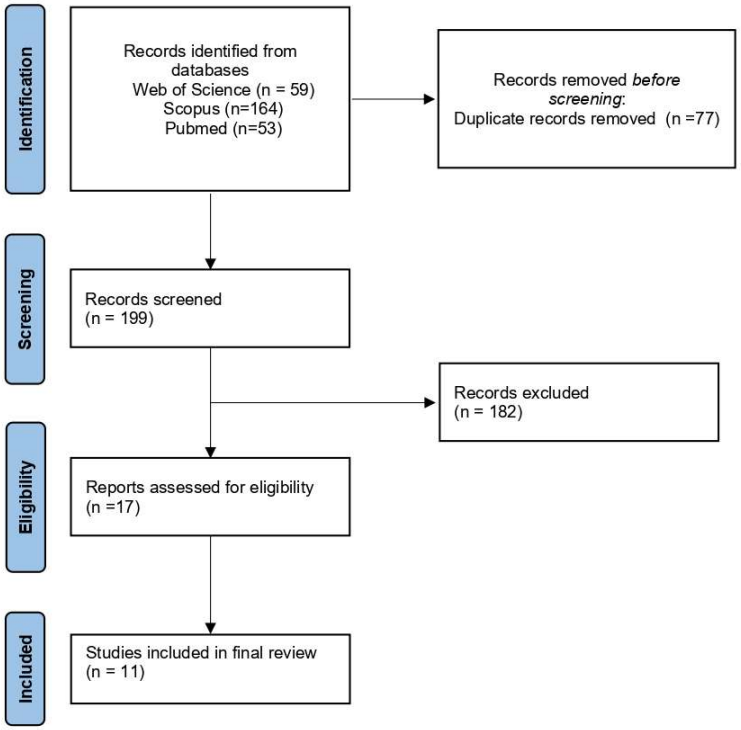

Figure 1. PRISMA flow diagram

Table 2: Description of each study and its main findings (animal studies)

Article	Experimental Model	Intervention	Receptor of interest	Methodology/ Measurements of effect	Main Findings
Ramirez et al, 2012	Mouse model of endotoxemic encephalitis (LPS)	JWH133 and O-1966 (CB2 selective agonists)	CB2R	Measurement of vascular permeability and leucocyte adhesion (in vivo) Blood-brain barrier permeability assessment Quantification of adhesion molecules	CB2R activation: -decreased leukocyte adhesion to brain endothelial cells -reduced the expression of endothelial adhesion molecules -reduced the blood-brain permeability and prevents its dysfunction
Kianian et al 2013	Rats (experimental endotoxemia)	FAAH inhibitor (URB597)	CB2R	Intravital microscopy, assessing functional capillary density and and leucocyte adhesion	FAAH inhibition: -prevented the LPS-induced increase in leukocyte adhesion in venules, partially reversed by CB2 antagonism. -increased the functional capillary density, irreversible by CB2 antagonism.
Toguri et al, 2015	Rats (LPS-induced toxemia)	WIN 55212-2 (CB1,CB2 and TRPV1 agonist)	CB2R and CB1R	Quantification of leucocyte adhesion and rolling. Measurement of arterial pressure and heart rate Assessment of iridial microvascular blood flow.	WIN 55212-2: -decreased leukocyte adhesion in LPS-treated rats, with complete reversal by CB2 antagonism. -improved iridial microvasculature blood flow, without reversal by CB1 or CB2 antagonists
Rom et al, 2013	Rat model of endotoxemic encephalitis (WT or CB2R knock out)	JWH 133, GP1a, AM1241 and O-1966	CB2R	Intravital video microscopy, with a previous treatment of collected leucocytes with calcein-AM.	CB2R agonism decreased leukocyte adhesion compared to untreated controls, partially reversed by CB2 antagonism. CB2R knockout leukocytes exhibited increased adhesion to the endothelium. WT leucocytes adhered more in CB2Rko mice, when compared to the WT mice.
Orliac et al, 2003	Experimental rat model of endotoxemia	Exogenous anandamide (CB1, CB2 and TRPV1 agonist)	Vanilloid receptors (not specified)	Blood pressure measurements Vascular reactivity assessment	Anandamide: -caused a concentration-dependent reduction of contractile responses to norepinephrine, that increased with a vanilloid agonist and decreased with vanilloid antagonist -caused a reduction of contractile responses to norepinephrine, potentiated by endotoxemia
Bányai et al 2023	Experimental rat model of LPS-induced endotoxemia	THC (CB1 and CB2 agonist)	CB1R and CB2R	Quantification of systolic and diastolic volumes Measurement of vascular reactivity Detection of systemic oxidative-nitrative stress and quantification of NO endothelial synthase	THC: -increased end-systolic volume in LPS-treated rats -prevented LPS-induced reduced endothelium-dependent relaxation of the aorta -reversed LPS-induced lipid oxidation and nitrative stress. -decreased endothelial NO synthase and COX2 density.
Singh et al, 2018	Experimental mouse model of sepsis (caecal ligation and puncture)	Inhibition of MAGL or of DAGL (enzymes responsible for degradation and anabolization of 2AG, respectively)	CB1R	Recording of the isometric tension of the thoracic aorta Quantification of CNR1 in the aorta	DAGL inhibitor improved the contractile response of aortic rings to noradrenaline in sepsis. CB1R antagonist increased the contractile response of aortic rings in sepsis. CB1R mRNA expression was increased during sepsis. Aortic contraction by K+ depolarizing solution was reduced when a CB1 antagonist was added in septic mice.
Rajesh et al, 2007	Experimental mice model of vascular inflammation (LPS)	HU-308, JWH-133 (CB2 agonists)	CB2R	Monocyte-endothelial adhesion assay Cell surface ICAM-1 and VCAM-1 expression assay	HU-308 and JWH-133 reduced the expression of ICAM-1 and VCAM-1. HU-308 reduced monocyte adhesion to endothelium.
Leite-Avalca et al, 2019	Experimental rat model of sepsis (CLP)	Rimonabant (CB1 inverse agonist)	CB1R	Dosage of LDH, lactate, glucose and CK-MB Tail Vasomotor Tone response by temperature measurement Isometric Tension Measurement	Rimonabant: -reversed CLP-induced alterations in glucose and lactate levels. -abolished the reduced tail temperature in CLP rats. -restored vascular reactivity to vasopressin.

Table 3: Description of each study and their main findings (*in vitro studies)*

Article	Experimental Model	Intervention	Receptor of interest	Measurements of effect	Main Findings
Ramirez et al, 2012	Primary human brain microvascular endothelial cells	JWH 133 and O-1966	CB2R	Assessment of vascular permeability and leucocyte adhesion Measurement of transendothelial electrical resistance Blood-brain barrier permeability assessment	CB2 concentration was increased in human brain endothelium by LPS. CB2R activation: -reduced the expression of endothelial adhesion molecules -increased the expression of tight-junction molecules CB2 concentration was increased in human brain endothelium by LPS.
Braile et al, 2020	Primary human neutrophils stimulated with LPS	ACEA (CB1 agonist) and JWH-133 (CB2 agonist)	CB1R and CB2R	Quantification of VEGF-A In vitro vascular permeability assay	CB agonists: -inhibited LPS-induced VEGF-A production, reduced the angiogenic response and reduced the vascular permeability induced by LPS. -did not affect the production or mRNA expression of CXCL8 or HGF nor the induced increase in CD66b or decrease of L-selectin
Wilhelmsen et al, 2014	Primary human endothelial cells of various tissues	WIN-55,212-2 (CB1, CB2 and TRPV1 agonist) N-arachidonoyl dopamine (NADA) (CB1 and TRPV1 agonist)	CB1R, CB2R, TRPV1, GPR18, GPR55	Quantification of IL-6, Il-8 and E-selectin. Leucocytes adhesion assay	NADA and WIN 55212-2 reduced IL-6 and IL-8 secretion from endothelial lung cells. NADA reduced the surface expression of E-selectin on endothelial cells. NADA reduced the adhesion of neutrophils to endothelial cell monolayers. CB1 and CB2 antagonists abated the effect of NADA on IL-8 levels at low doses..

Regarding study quality, all included articles achieved a quality score above 75%. However, the criterion with the lowest scores was “evaluation by independent observers, blinding, and evidence of independent repetitions in data collection”, suggesting methodological limitations that could introduce bias. This underlines a need for greater rigor in experimental design and validation in future studies exploring the role of the endocannabinoid system in sepsis.

The included studies predominantly employed experimental models using animal or cellular systems, wherein cannabinoid compounds were administered. While these models provide valuable mechanistic insights, their translational applicability remains a concern. As sepsis a characterized by complex organ failure and high mortality risk, these features were challenging to replicate in experimental settings, which potentially limits clinical extrapolation.

In this study, results are presented in separate tables for animal studies (Table 1) and *in vitro* studies (Table 2) to clarify and ease data interpretation. The fundamental differences between these experimental models justify distinct presentation, which allows for a more transparent evaluation of the results of each model. *In vitro* studies provide mechanistic insights at cellular level, while animal studies contribute to a more systemic understanding of these effects.

Among the included studies, nine focused on classical cannabinoid receptors, CB1 and CB2, while two explored the vanilloid receptor's role. In microvascular dysfunction during sepsis. Specifically, four studies examined CB2 receptor activity alone, two focused on CB1, and three investigated both receptors in combination or alongside other receptors of interest. This distribution is particularly relevant given the distinct functional roles of these receptors. CB2 receptor activity is primarily associated with anti-inflammatory pathways and immune regulation, whereas CB1 receptor activity is more closely linked to cardiovascular regulation, including vasodilation and hemodynamic responses. The differential research focus may also reflect the anatomical localization of each receptor.

In this study, the results are (besides their localization in Table 2 and 3) explicitly placed into the discussion to provide a more comprehensive and contextualized interpretation of the results. This allows a more direct comparison between our data and existing literature, facilitating the establishment of similarities, discrepancies, and potential mechanisms.

Discussion

This systematic review analyzed 11 articles, including both animal and human *in vitro* models, reflecting the slow progress and limited funding of this subfield. Despite these constraints, some conclusions can be drawn regarding the modulatory role of endocannabinoids in the microvascular pathophysiology of sepsis. This modulation is mediated through various molecular cascades and receptor-specific effects (despite some variability, within the same receptor, by using different activating substances), particularly CB1 and CB2 and vanilloid receptors, discussed individually in this discussion. Additionally, the unique properties of THC, a partial agonist of both CB1 and CB2, warrant specific considerations due to its distinct pharmacological profile.

CB1

CB1 is the most well-studied receptor of the endocannabinoid system, with multiple central and peripheral effects, such as in inflammatory conditions and pain modulation. Beyond these well-documented roles, it has been implicated in vascular tone regulation and metabolism, through its presence in endothelial and smooth muscle cells, which, despite expressing lower CB1 levels, retain functional activity [31]. In fact, Wilhelmsen et al. (2014) detected *CNR1* (the mRNA encoding CB1R) in various endothelial tissues, providing key experimental validation and supporting its physiological relevance (Supplementary Table 1). Notably, this study remains the only one to directly confirm CB1 receptor presence in these cells.

As outlined in the results' section, 6 articles investigated the impact of CB1 modulation in septic models (Braile et al. 2020; Toguri et al. 2015; Wilhelmsen et al. 2014; Bányai et al. 2023; Singh et al. 2018; Leite-Avalca et al. 2019). Collectively, these articles indicate a sturdy trend towards beneficial effects of endocannabinoid modulation in the physiological mechanisms of sepsis, especially in the contractile response and blood pressure regulation (Toguri et al., 2015, Singh et al. 2019 and Leite-Avalca et al. 2019). Kianian et al. (2013) proposed a non-CB2 mediated effect on vascular contractility, while Braile et al. (2020) explored CB1's role in angiogenesis and vascular permeability. Addicionally, Leite-Avalca et al. (2019) demonstrated that CB1 receptor antagonism decreased markers of organ dysfunction. However, findings on CB1 receptor expression remain inconsistent, with studies reporting both upregulation and downregulation. These differences may be attributed to tissue-specific responses, stage of sepsis at the time of measurement and, most likely, the complex pharmacological behavior of THC, which was underexplored (Bányai et al. 2023). These variations could significantly impact the interpretation and clinical application of these findings.

The results of this review align with findings in different settings, adding a layer of credibility to these results. Similarly to the CB1-mediated hypotensive effects shown by Bányai et al. 2023, Toguri et al. 2015, Singh et al. 2018 (and suggested by Kianian et al. 2013), other studies have proved this effect in hemorrhagic shock [32], after myocardial infarctions [33] and in normal physiological conditions, previously described by Malinowska et al. (2017) [8], addressing the trifasic effect on blood pressure. Despite this, Leite-Avalca et al. (2019) reported a rimonabant-dependent increase in tail temperature, suggesting a vasodilatory effect of CB1 antagonism. Furthermore, Bernardelli et al. (2016) suggests that the vascular responses vary depending on the specific tissue examined, the timing and type of inflammatory insult. These findings imply that CB1's role extends beyond simple hypotensive effects, instead differentially modulating blood vessel contractility. This is clinically significant, as arterial blood pressure regulation remains one of the most complex yet critical aspects of sepsis management, with multiple therapeutic strategies available to the physicians.

A compelling finding from this review is CB1's potential role in modulating angiogenesis and vascular permeability, two processes increasingly recognized as possible targets in sepsis. While the role of vascular endothelial growth factor (VEGF) remains debated, its dysregulation can severely impair endothelial function. Insufficient VEGF levels contribute to endothelial apoptosis and reduced survival, while excessive VEGF promotes inflammation, vascular dysfunction and leucocyte adhesion. Braille et al. (2020) demonstrated a partial CB1-mediated effect on VEGF levels, coinciding with ecreased vascular permeability. This observation is noteworthy, as such effect are more commonly attributed to CB2 agonism. [35] These findings highlight the

complexity of endocannabinoid signaling in sepsis and underscore the need for further experimental research.

CB2

The CB2 receptor is often described as the "shy" counterpart of CB1, sharing 44% overall genomic identity [36] while being much less prevalent in the tissues where CB1 is predominant, the CNS, where it can be upregulated in neuroinflammatory states in glial cells [37]. CB2 is more abundant in immune and lymphoid tissues (e.g., thymus, spleen, and peripheral blood mononuclear cells). Additionally, low levels of CB2 expression have been detected in cardiomyocytes and endothelial cells [24], thereby further broadening the receptor's physiological relevance. However, Wilhelmsen et al. (2014) highlighted a critical issue: when trying to detect CB2 receptor, the evidence was faint and inconsistent (Supplementary Table 1). This observation suggests that, under some experimental conditions, CB2's presence is ambiguous, in contrast to earlier studies reporting robust expression. In essence, the reliability of detecting CB2 appears to depend on the specific methods used and further research employing diverse methodological approaches is essential to confirm CB2's expression in endothelial cells and fully unravel its physiological significance.

The CB2-related information provided by these articles (4 that studied it individually and another 4 where it is evaluated along another receptor) consistently indicates that stimulation of the CB2 receptor significantly reduces leukocyte adhesion to the endothelial walls.

Ramirez et al. (2012) and Rajesh et al. (2007) reported a reduction of the expression in intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), proteins that play a role in leukocyte adhesion and are present in both endothelial and immune cells. In contrast, Braile et al. (2020) reported no measurable effect on adhesion molecules that are exclusively expressed by immune cells (e.g., CD66b and L-selectin) (Supplementary Table 1). These discrepancies suggest that the anti-adhesive effects of CB2 activation may primarily be mediated via its action on endothelial cells. Despite this, Rom et al. (2013) showed that leukocytes derived from CB2 knockout (CB2ko) rats adhered more strongly to the endothelium compared with those from wild-type rats, whereas wild-type leukocytes displayed increased adhesion when interacting with the endothelium of CB2ko rats. Collectively, these observations tend to suggest that CB2 modulates cellular adhesion processes in both immune and endothelial compartments, despite previous conflicting reports.

In parallel with these findings, CB2 agonism also enhanced endothelial-barrier function. Ramirez et al. 2012 demonstrated that CB2-dependent mechanisms attenuated LPS-induced endothelial permeability, an effect accompanied by the upregulation of tight-junctions' proteins. Similarly, Braile et al. (2020) observed that treatment with the CB2 agonists ACEA and JWH-133 reduced

vascular permeability to polymorphonuclear cells; this effect was partially reversible by both CB1 and CB2 antagonists. These findings are particularly relevant in neuroinflammation, where disruption of the blood-brain barrier poses a significant risk to the central nervous system homeostasis.

Combined effect of THC in CB1 and CB2

The potential combined effects of tetrahydrocannabinol (THC), a phytocannabinoid extracted from *Canabis sativa* with CB1 and CB2 partial agonism activity, on both these receptors have also been explored in sepsis-induced circulatory dysfunction. One study (Bányai et al. 2023) reported that THC administration resulted in a reduction of several inflammatory markers, including indices of lipid oxidation, oxidative and nitrative stress, as well as DNA damage , even though it was associated with a reduction in CB1 receptor expression. Moreover, while some studies indicate that THC may induce endothelial-dependent relaxation in thoracic aorta segments, thus suggesting vasodilatory effects, similar end-diastolic volumes and cardiac outputs in LPS-treated groups — regardless of THC administration — suggest that THC does not effectively counteract LPS-induced vasoconstriction. Therefore, the evidence does not conclusively support a vascular benefits of THC in sepsis. Nonetheless, the divergent findings regarding THC's inflammatory effects warrant further investigation, which may be more appropriately addressed in a subsequent, dedicated review, as it does not fall within the scope of this review.

Vanilloid receptors

Interest in vanilloid receptors has grown markedly in recent decades. They are responsible for the peripheral transduction of thermal stimuli and nociception, being gated by physical (such as heat, aggression, etc) and chemical stimuli (protons, their more classical agonists capsaicin, menthol, mustard, camphor) and endocannabinoid agonists. TRPV1 has garnered particular attention in the endocannabinoid field because of the similarities between endogenous CB1 agonists and TRPV1 agonists (anandamide, for example, is an agonist for both receptors, despite having a greater affinity for the CB1 receptor; this happens with NADA and arachidonyl ethanolamine, in a similar way) [38]. This pharmacological overlap has prompted studies investigating TRPV1's potential role in modulating the endocannabinoid system.

Wilhelmsen et al. (2014) reported that administration of NADA and WIN-55,212-2 significantly reduced interleukin-8 (IL-8) levels and decreased the superficial expression of E-selectin, a molecule critical for endothelial leucocytes recruitment that can impair vascular flow and contribute to vascular dysfunction. Importantly, these anti-inflammatory effects were reversed by a TRPV1 antagonist, suggesting that this receptor may confer protection under basal conditions,

but adopt a proinflammatory role upon activation. Furthermore, the inhibitory impact of NADA on inflammatory markers was diminished in the presence of CB1 and CB2 antagonists, implying an interaction between these receptors in regulating inflammation. Future studies that test combined antagonism of CB1, CB2, and TRPV1 on leukocyte adhesion would help clarify the interplay between these receptos, as previous evidence indicates that TRVP1 activation may enhance leukocyte-endothelial adhesion [33].

In contrast, Orliac et al. (2003) examined the contractile effects of anandamide in mesenteric beds and found no significant difference in norepinephrine-induced contraction between septic and control tissues, despite an increase in inducible nitric oxide synthase (iNOS) activity (Supplementary Table 1). This observation may indicate limitations in the experimental design or an eveluation at an early time point that failed to capture diminished adrenergic responses — and effect that has been documented in other studies (Mitolo-Chieppa et al. 1996 [40] and Singh et al. 2018). Nevertheless, the study highlights the vasodilatory properties of anandamide, which are potentiated by endotoxemic conditions and modulated via TRPV1. Such findings suggest that modulation of TRPV1-mediated vasodilation may represent a viable therapeutic strategy for addressing septic vascular dysfunction.

Study limitations

Limitations regarding inflammatory models and translational relevance

A notable limitation of this review is the heterogeneity of inflammatory models employed across the included studies. Several investigations focused on systemic inflammatory responses that, although partially analogous, do not fully replicate sepsis. For example, two studies examined neuroinflammation and one used alternative inflammatory stimuli (including TNF-alfa-based models) alongise LPS. Moreover, many articles excluded from this review lacked a septic/endotoxemic model where endocannabinoid modulation was assessed, suggesting a potential research gap despite the extensive investigation of endocannabinoids in inflammation. Additionally, only one study (Singh et al. 2018) reported a nonzero mortality rate, casting further doubt on whether a genuine septic state was established in the experimental models. Although yielding intriguing conclusions, its limitations — including the lack of human-based clinical evidence and the variability of experimental models — underscore the need for standardized conditions to better elucidate the complex and sometimes contradictory vascular effects of the endocannabinoid system.

Limitations regarding risk of bias assessment

Another limitation is the absence of a formal risk-of-bias evaluation. Although tools such as the SYRCLE tool and CAMARADES checklist exist for animal studies, they do not fully reflect

typical reporting practices in this field. Notably, the studies included in our review dido not report critical parameters — such as allocation concealment, random housing, or adequate blinding of caregivers and outcome assessors — making it difficult to assess potential biases uring these instruments. We suggest that this omission reflects a misalignment between current risk-of.bias tools and actual reporting standards rather than poor study quality. Nevertheless, for the sake of transparency and comparability, further research should strive to implement and report these methodological safeguards.

Conclusion

This review demonstrates that the endocannabinoid system represents a promising target for sepsis mangement, as its modulation — via CB1, CB2, and TRPV1 receptors — exerts significant effects on key vascular functions, including leukocyte adhesion, vascular tone, endothelial barrier integritu and even angiogenesis. However, the translation of these findings is limited by the heterogeneity of septic and inflammatory models, the scarcity of clinical data, and methodological inconsistencies, highlighting the need for standardized experimental protocols and rigorous bias assessment in future research.

Statements and Declarations

Funding

The authors declare that no funds, grants, or other support were received during preparation of this
manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Authors Contributions

All authors contributed to the design and conceptualization of the study. FMVR had a major role in the acquisition, analysis, and interpretation of data, drafting the first draft of the manuscript. EM and MAVC also contributed for the acquisition, analysis, and interpretation of data and reviewed the draft in its posterior versions. All authors read and approved of the final manuscript.

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Supplementary Material

Supplementary Table 1: Complete collection of the results and methodology of all included studies.

Study	Authors	Experimental Model	Intervention	Receptor of interest	Methodology/ Measurements of effect	Main Findings
Activation of Cannabinoid Receptor 2 Attenuates Leukocyte-Endothelial Cell Interactions and Blood-Brain Barrier Dysfunction under Inflammatory Conditions	Ramirez, Hasko, Skuba	Mouse model of endotoxemic encephalitis (LPS) Primary human brain microvascular endothelial cells	JWH133 and O-1966	CB2R	Intravital microscopy (cranial window) to access vascular permeability and leucocyte adhesion (*in vivo*) Measurement of transendothelial electrical resistance Adhesion assays Immunohistochemistry and immunofluorescence Blood-brain barrier permeability assessment	- CB2R is overregulated, in the brain, by LPS. - Selective CB2R activation significantly reduces leucocyte adhesion, effect nearly totally reversed by a selective antagonist -Complete resolution of inflammation by LPS took significantly longer in CB2 knocked-out animals than for the wild-type animals. -CB2 agonists had no effect on the number of endothelial-leucocyte interactions in CB2ko animals. -Similar results were obtained when the type of vasa (surface pial vessels vs brain parenchymal vessels) was changed, for the measurement of leucocyte adhesion in the LPS+CB2 agonist group. -CB2 agonists reduce the expression of adhesion molecules (ICAM-1 and VCAM-1) in endothelial brain cells, that are upregulated by LPS - CB2 agonists attenuate the degree of permeability induced by LPS insult - CB2 activation improves BBB function by promoting a tight-junction proteins (claudin-5 and ocludin) increase, when compared to the measured levels in LPS-treated animals.

LPS-mediated neutrophil VEGF-A release is modulated by cannabinoid receptor activation	Braile, Cristinziano, Marcella	Primary human neutrophils stimulated with LPS	ACEA (CB1 agonist) and JWH-133 (CB2 agonist)	CB1R and CB2R	ELISA assays mRNA extraction and quantitative PCR analysis Apoptosis assay Flow cytometry *In vitro* Matrigel angiogenesis assay, with quantification of tubules and analysis of tubule length *In vitro* vascular permeability assay,	- CB agonists inhibited the LPS-induced production of VEGF-A, reaching a maximum effect at 16h. This effect was partially reversed by CB1 and CB2 antagonists. - The CB agonists also diminished VEGF-A mRNA expression. - CB agonists had no effect in production and mRNA expression of CXCL8 or HGF. - Cannabinoid agonists had no effect on the increase of CD66b expression or in the decrease of L-selectin, caused by LPS. - The presence of ACEA and JWH-133 reduced the angiogenic response of LPS-activated PMNs (reduced tubule length and number of tubules), partially reversed by the addition of CB antagonists. - ACEA and JWH-133 reduced the vascular permeability induced by supernatants of LPS-induced PMNs. This effect was, in part, reversible by CB1 and CB2 antagonists.
Inhibition of endocannabinoid degradation in experimental endotoxemia reduces leukocyte adhesion and improves capillary perfusion in the gut	Kianian, Al-Banna, Kelly	Rats (experimental endotoxemia)	FAAH inhibitor (URB597)	CB2R	Intravital microscopy, assessing functional capillary density and leucocyte adhesion Degree of histological injury, observed by light microscopy	- Inhibition of the FAAH prevented the LPS-induced increase in leukocyte adhesion in V1 and V3 venules, reversible in part by the addition of a selective antagonist of CB2R - URB597 increased the functional capillary density of mucosal and circular muscular layers of the intestines, without reversion by the CB2R antagonist - FAAH inhibition showed an absence of effect in vital parameters and in histological evaluation.
Cannabinoid 2 Receptor activation reduces leukocyte adhesion and improves capillary perfusion in the iridial	Toguri, Moxsom, Szczesniak	Rats (LPS-induced toxemia)	WIN 55212-2	CB2R and CB1R	Intravital microscopy of randomly chosen iridial capillaries, used to quantify leucocyte adhesion and rolling.	- WIN 55212-2 significantly decreased adhesion of leucocytes in LPS-treated rats, with abolishment of effect with CB2R antagonist, AM-630, in vessels larger than 25µm.

microvasculature during Systemic inflammation					Measurement of arterial pressure and heart rate Laser Doppler flowmetry to assess the iridial microvascular blood flow.	- The addition of the cannabinoid agonist didn't cause any effect in hemodynamics or number of rolling leucocytes. - Cannabinoid treatment improved the iridial microvasculature blood flow considerably, without a significant difference caused by any antagonists
The Endocannabinoid/Endovanilloid *N*-Arachidonoyl Dopamine (NADA) and Synthetic Cannabinoid WIN55,212-2 Abate the Inflammatory Activation of Human Endothelial Cells	Wilhelmsen, Khakpour, Tran	Primary human endothelial cells of various tissues (umbilical vein, brain microvasculature, liver sinusoidal microvascular, microvascular lung and coronary endothelial cells) induced with either FSL-1, LPS or TNFα.	WIN-55,212-2, *N*-arachidonoyl dopamine (NADA), 2AG, CBD, abnormal cannabidiol, arachidonoyl-2'-chloroethylamide, N-arachidonoylethanolamide, anandamide, cannabidiol, capsaicin, HU-308, NADA, N-arachidonoyl glycine and WIN55,212-2.	CB1R, CB2R, TRPV1, GPR18, GPR55	Immunoblots ELISA Crystal Violet Assay MTT Assay Flow Cytometry Quantitative Real Time PCR Neutrophil Adhesion assay Electric Cell-Substrate Impedance Sensing Antagonist/Inverse Agonist Assays Calcium Influx assay	- CNR1 (mRNA of CB1R), GPR18 and TRPV1 were detected in all cell models studied (mentioned in "Experimental Model"). CNR2 (mRNA of CB2R) was detected in almost all tissues (not detected in human microvasculature endothelial cells (HMVEC) of lung and of liver), only when a second assay was made, with primers for different exons. GPR55 was detected in all cell models only when a different primer was used. - NADA and WIN55,212-2 were the only cannabinoid (and non-cannabinoid, including the capsaicin) that reduced IL-6 and IL-8 secretion from endothelial lung cells stimulated by LPS and FSL-1, without compromising cellular viability. - The effects of CB1 and CB2 antagonists had minimal results in lowering the IL-8 levels after WIN55,212-2. - The presence of TRPV1 antagonist together with WIN55,212-2 exacerbated the benefit reported but, when in the absence of WIN55,212-2, the antagonist alone increased IL-8 levels. - Only NADA (and not its chemical constituents) reduce the expression of IL-8 - NADA reduces the surface expression of E-selectin on endothelial cells activated with LPS or FSL-1. - NADA dose-dependently reduces the adhesion of primary human neutrophils to HMVEC-lung monolayers. - Treatment with either WIN55,212-2 and NADA failed to

						alter the increased endothelial permeability - CB1 and CB2 antagonists strongly and significantly abated the effect verified in the reduction of IL-8 levels by NADA, in low doses. In high doses, the effect was contrary, aggravating the effect of NADA. - The presence of TRPV1 antagonist, along with NADA reversed the benefit reported but, when in the absence of NADA, the antagonist alone augmented IL-8 levels. - Endothelial cells possess the enzymatic machinery to produce NADA but technical analysis failed to detect endogenous NADA in human endothelial cells lysates.
Selective Activation of Cannabinoid Receptor 2 in Leukocytes Suppresses Their Engagement of the Brain Endothelium and Protects the Blood-Brain Barrier	Rom, Zuluaga-Ramirez, Dykstra	Experimental rat model of endotoxemic encephalitis (WT or CB2R knock out)	JWH133, GP1a, AM1241 and O-1966	CB2R	Intravital video microscopy, with a previous treatment of collected leucocytes with calcein-AM.	- CB2R agonism decreased treated injected leukocyte adhesion when compared to the absence of treatment and to autologous leucocytes; this effect was only partially reversed by an inverse agonist (SR144528). - CB2Rko leucocytes exhibited a an important increase in adhesion to the endothelium when compared to the WT counterparts - WT leucocytes showed an increase in adhesion in CB2Rko mice, when compared to the WT mice.
Potentiation of Anandamide Effects in Mesenteric Beds Isolated from Endotoxemic Rats	Orliac, Peroni, Celuch	Experimental rat model of endotoxemia, with repeated contractions of mesenteric vascular beds with norepinephrine.	Exogenous anandamide	Vanilloid receptors (not especified)	Blood pressure measurements with a tail-cuff method Nitric oxide synthase activity quantification Vascular reactivity assessment	- No differences were observed in NE-induced mesenteric beds contraction between LPS-treated rats compared with controls. - Anandamide caused a concentration-dependent reduction of the contractile responses to NE. These relaxations were significantly potentiated in mesenteric beds 6h after exposition to LPS. - Capsazepine (vanilloid receptor antagonist) significantly attenuated the effect of anandamide on the reduction of NE-induced contractions, while capsaicin (vanilloid receptor

						agonist) increased it. - No differences were observed in the effect of anandamide in mesenteric beds contractions after endothelium removal. - The anandamide amidase inhibitor addition (enzyme responsible for the degradation of the endocannabinoid) caused a significant reduction of anandamide effects on mesenteric beds isolated from LPS-treated rats.
Delta-9-Tetrahydrocannabinol conserves Cardiovascular Functions in a rat model of endotoxemia: Involvement of endothelial molecular mechanisms and oxidative-nitrative stress	Bányai, Répás, Miklós	Experimental rat model of LPS-induced endotoxemia	THC	CB1R and CB2R	Echocardiography Examination of blood pressure and left ventricular function Measurement of vascular reactivity in isolated aortic rings of rats Malonyl-dialdehyde assay (detection of systemic oxidative-nitrative stress) Immunohistochemical staining	- End-systolic volume, in particular, was increased in LPS+THC treated rats, compared to positive controls (only LPS-treated). -Other cardiac functions evaluated (heart rate, inotropy and lusitopy) were not affected by the intervention. - LPS caused a reduced endothelium-dependent relaxation of the thoracic aorta segments, after epinephrine contraction, that was prevented by THC. - Lipidic oxidation and nitrative stress, greatly augmented in LPS treated rats, was reversed with the treatment with THC. DNA damage and oxidative stress, while elevated with LPS+THC, did not have a significant increase compared to controls. - The density of endothelial NO synthase was not affected by LPS but was significantly decreased with THC treatment, with a similar result with the density of COX2 - The density of cGMP was decreased in both LPS-only and LPS+THC groups. - CB1R density in the thoracic aorta was diminished in LPS and in LPS+THC groups, while CB2R density showed only a declining tendency in LPS group, with the LPS+THC group showing a similar abundance to controls.

Endocannabinoid-mediated modulation of Gq protein-coupled receptor mediates vascular hyporeactivity to nor-adrenaline during polymicrobial sepsis	Singh, Sharma, Nakade	Experimental mouse model of sepsis (caecal ligation and puncture)	Inhibition of MAGL or of DAGL (inhibition of the degradation and the anabolization of 2AG, respectively)	CB1R	Recording of the isometric tension of the thoracic aorta Quantitative Reverse Transcriptase polymerase chain reaction (qRT-PCR)	- Noradrenaline-induced contraction of the aortic rings was significantly reduced in early and late sepsis (6 and 20h, respectively) and also when compared to controls. - The concentration- response curve (CRC) of NA was shifted to the left in the presence of a DAGL inhibitor (KT 109), in sham-operated mice. - The CRC of NA, in sham-operated mice, was significantly shifted to the right in the presence of a MAGL inhibitor (JZL 184). - KT 109 improved the contractile response of aortic rings of mice to NA in early and late sepsis. - JZL 184 didn't alter in a significant way the CRC of NA in septic mice. - The response of aortic rings to NA wasn't altered by rimonabant, either in So or septic mice. - AM 251(selective peripheral CB1R antagonist) significantly increased the contractile response of aortic rings in septic mice. However, it failed to have the same effect in SO mice. - mRNA expression of CB1R in thoracic aorta rings during both phases of sepsis was importantly increased. - K+-depolarizing solution-induced aortic contraction was significantly reduced when AM 251 was used in septic mice, not achieving the same result in sham-operated mice. - CaCl2-induced contraction wasn't affected by the addition of AM 251 in both septic or sham-operated mice.
CB2-receptor stimulation attenuates TNF-α-induced human endothelial cell activation, transendothelial	Rajesh, Mukhopadhyay, Bátkai	Experimental mice model of vascular inflammation	HU-308, JWH-133	CB2R	Monocyte-endothelium adhesion assay (with *ex vivo* aortic segments Cell surface ICAM-1 and VCAM-1 expression assay	- HU-308 and JWH-133 significantly reduced the expression of ICAM-1 and VCAM-1, reversed by the addition of a CB2R agonist (AM-630). - HU-308 significantly reduced the monocyte adhesion to

migration of monocytes and monocyte-endothelial adhesion		(endotoxemia by LPS injection)				endothelium of aortic segments, with a marked reversion of effect with AM-630.
Cannabinoid CB1 Receptor Antagonist Rimonabant Decreases Levels of Markers of Organ Dysfunction and Alters Vascular Reactivity in Aortic Vessels in Late Sepsis in Rats	Leite-Avalca, Staats, Verona	Experimental rat model of sepsis (CLP)	Rimonabant	CB1R	Evaluation of Markers of Organ Failure (dosage of LDH, lactate, glucose and CK-MB) Evaluation of Hematological Parameters (blood cells counting) Nitrate/Nitrite Assay Tail Vasomotor Tone response (Temperature monitoring by infrared camera) Isometric Tension Measurement	-The significant alterations caused by CLP of glucose and lactate were reversed by rimonabant, to equivalent levels of those of sham-operated rats, at 6 and 20h after the procedure. - LDH was only brought to comparable levels of the controls at 24h and CK-MB at 6h after the caecal puncture. - Rimonabant had no effect on blood cell countings of septic rats - Rimonabant had no effect on plasma nitrate/nitrite levels - The reduced tail temperature in CLP rats was completely abolished in rimonabant-treated rats. - The vascular reactivity of rimonabant treated rats was identical to sham-operated rats, when stimulated with cumulative concentrations of vasopressin, in rats with 24h of septic evolution. - Phenylephrine induced-contraction wasn't significantly affected by rimonabant treatment, nor was the high-concentrated KCl solution contraction.

The PRISMA Statement: Checklist of items that should be included in reports of s*ystematic reviews*

Section/topic	#	Checklist item	Reported on page and paragraph/ table #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both. **- MANDATÓRIO**	Page 2: "**A systematic approach to the role of the endocannabinoid system in the microvascular dysfunction of sepsis**
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. **– SEGUIR RECOMENDAÇÕES DA REVISTA**	Page 2 **Introduction**: The endocannabinoid system plays a key role in regulating inflammation, immune responses, and vascular homeostasis. In recent years, it has been extensively studied as a promising therapeutic target for a variety of pathological conditions, including inflammatory and autoimmune diseases. Sepsis is a life-threatening condition driven by immune dysregulation and exaggerated inflammatory response to infection, leading to widespread endothelial dysfunction and microvascular impairment, that drive multi-organ failure. However, the interaction between microvascular dysfunction in sepsis and the endocannabinoid system remains poorly explored in scientific literature. **Objective and Methods**: A systematic review was conducted to analyze the relationship between the endocannabinoid system and microvascular dysfunction in sepsis. A literature search was performed using PubMed, Scopus, and Web of Science databases, selecting studies that employed experimental models of sepsis in which interventions targeted endocannabinoid/cannabinoid modulation and evaluated microvascular and/or platelet function. The risk of bias and methodological quality of the studies were evaluated using a standardized scale developed by Cosme D. et al.. **Results**: A total of 11 studies were included, 9 of which were *in vivo* studies in animal models and 2 *in vitro* studies in human cells. The most frequently investigated molecular target was the CB2 receptor (9 studies), whose activation was consistently associated with a reduced leukocyte adhesion, suggesting a protective effect against microvascular inflammation. Furthermore, a decrease in VEGF production and anti-angiogenic effects were observed with the activation of CB1 and CB2 receptors. The CB1 receptor, in turn, was found to influence vascular contractile responses in a context-dependent manner.

			Conclusion: Endocannabinoid system modulation emerges as a promising therapeutic approach for sepsis, particularly in preventing microvascular complications associated with the disease and modulating blood pressure. Therefore, evaluating this potential in humans is essential to validate the translational relevance of these findings and their clinical applicability. : “
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. **– MANDATÓRIO** ***O rationale corresponde à justificação da importância da revisão sistemática***	Page 4: “Given the ECS’s role in maintaining vascular integrity and the critical involvement of endothelial dysfunction in the transition of sepsis to septic shock, cannabinoid-mediated modulation of endothelial responses during warrants further exploration. However, the precise mechanisms by which CB1 and CB2 receptor activation influence vascular responses in sepsis remain poorly understood. This review aims to synthesize the current evidence on the link between the endocannabinoid system and vascular dysfunction in sepsis, highlighting its potential as a therapeutic target for minimizing sepsis-related complications.
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). **- MANDATÓRIO**	Page 4: “This review aims to synthesize the current evidence on the link between the endocannabinoid system and vascular dysfunction in sepsis, highlighting its potential as a therapeutic target for minimizing sepsis-related complications
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. **– FACULTATIVO**	Not available.

Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. **– MANDATÓRIO** ***É altamente recomendado, de acordo com as boas práticas da Cochrane, que não sejam aplicados critérios de exclusão baseados na língua e/ou data de publicação dos estudos.***	Page 4: "This systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A meta-analysis was not performed. We evaluated studies investigating the effects of cannabinoids on vascular homeostasis and hemostasis. The target population included experimental models of sepsis that incorporated an infectious component or an infection-based systemic inflammatory response (LPS). Eligible models included live animal models, human models, and *in vitro* studies. The intervention of interest was any modulation of the endocannabinoid system (e.g., antagonism, agonism, enzyme activity variations) within the context of sepsis. No restrictions were applied to comparators. The primary outcomes were endothelial integrity, platelet function and overall vascular competence. We included all parameters measuring these functions as reported in the selected studies. A secondary outcome was the potential therapeutic application of endocannabinoid ligands for sepsis and septic shock, particularly targeting platelet and endothelial dysfunction. Eligible study designs included randomized controlled trials, animal model experiments, and in vitro studies. No restrictions were placed on language or publication year. A comprehensive literature search was performed in PubMed (1997–present), Web of Science (1999–present), and SCOPUS (1980–present), between October 28 and November 1, 2024, with the final search conducted on November 2, 2024. The following search query was applied across all platforms: [[cannabinoids] OR [endocannabinoids] OR [cannabinoid receptor]] AND [[inflammation] OR [systemic inflammatory response syndrome] OR [sepsis]] AND [[microvessels] OR [endothelium] OR [blood platelets] OR [hemostasis]] [All Fields]. Study selection occurred in two phases, conducted independently by two reviewers. Initially, titles and abstracts were screened for relevance. In the second phase, full-text articles were reviewed to determine eligibility. Data extraction was performed manually and independently by both reviewers, with disagreements resolved by discussion and consensus. Extracted data included: study authorship, title, publication year, intervention details, study population or model, methodology, cannabinoid receptor of interest, and main findings.
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. **– MANDATÓRIO** ***Em consonância com as boas práticas da Cochrane, é mandatório que se verifique***	Page 5: "A comprehensive literature search was performed in PubMed (1997–present), Web of Science (1999–present), and SCOPUS (1980–present), between October 28 and November 1, 2024, with the final search conducted on November 2, 2024. The following search query was applied across all platforms: [[cannabinoids] OR [endocannabinoids] OR [cannabinoid receptor]] AND [[inflammation] OR [systemic inflammatory response syndrome] OR [sepsis]] AND [[microvessels] OR [endothelium] OR [blood platelets] OR [hemostasis]] [All Fields].

		pesquisa em pelo menos duas bases de pesquisa bibliográfica (idealmente, deverão ser pesquisadas duas bases generalistas e uma específica da área). No caso de revisões sistemáticas de estudos experimentais/ensaios clínicos aleatorizados, é altamente recomendado que uma das bases pesquisadas corresponda à CENTRAL ou a bases de ensaios clínicos como a ClinicalTrials.gov. ***Estudos de revisão da literatura em que a pesquisa decorra numa única base de dados não serão classificados como revisões sistemáticas.***	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. **– MANDATÓRIO** ***A query de pesquisa deve ser obrigatoriamente disponibilizada. A***	Page 5: "The following search query was applied across all platforms: [[cannabinoids] OR [endocannabinoids] OR [cannabinoid receptor]] AND [[inflammation] OR [systemic inflammatory response syndrome] OR [sepsis]] AND [[microvessels] OR [endothelium] OR [blood platelets] OR [hemostasis]] [All Fields].

		utilização de filtros de pesquisa da InterTASC é altamente recomendada (https://sites.google.com/a/york.ac.uk/issg-search-filters-resource/home)	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). **– MANDATÓRIO** ***As fases de selecção dos estudos primários devem ser descritas. Em consonância com as boas práticas da Cochrane, é mandatório que o processo de selecção envolva duas fases (fase de rastreio, em que os registos são seleccionados por título e abstract, e fase de inclusão, na qual se procede à leitura integral dos full texts). Em cada uma destas fases, o processo de selecção deve mandatoriamente envolver dois***	Page 5: "Study selection occurred in two phases, conducted independently by two reviewers. Initially, titles and abstracts were screened for relevance. In the second phase, full-text articles were reviewed to determine eligibility. Data extraction was performed manually and independently by both reviewers, with disagreements resolved by discussion and consensus. Extracted data included: study authorship, title, publication year, intervention details, study population or model, methodology, cannabinoid receptor of interest, and main findings."

		investigadores actuando de forma independente.	
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. **– MANDATÓRIO** ***Trata-se de descrever de que forma se procedeu à extracção de dados dos estudos primários. Em consonância com as boas práticas da Cochrane, tal processo deverá envolver dois investigadores de forma independente.***	Page 5: "Data extraction was performed manually and independently by both reviewers, with disagreements resolved by discussion and consensus. Extracted data included: study authorship, title, publication year, intervention details, study population or model, methodology, cannabinoid receptor of interest, and main findings."
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. **– MANDATÓRIO** ***Trata-se de descrever as variáveis para as quais foi obtida informação.***	Page 5: "Data extraction was performed manually and independently by both reviewers, with disagreements resolved by discussion and consensus. Extracted data included: study authorship, title, publication year, intervention details, study population or model, methodology, cannabinoid receptor of interest, and main findings

Risk of bias in individual studies / Risk of bias across studies	12/ 15	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. **– MANDATÓRIO** ***Em todas as revisões sistemáticas, deverá existir um processo de avaliação da qualidade dos estudos primários. No caso de revisões sistemáticas de estudos experimentais/ensaios clínicos aleatorizados, a aplicação dos critérios de risco de viés (Risk of Bias) da Cochrane é altamente recomendada. No caso de revisões sistemáticas de estudos observacionais, poderão ser seguidos os critérios ROBINS ou os critérios dos National Institutes of Health (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools).***	Page 5: "To assess methodological quality, we applied a 14-item quality checklist developed by Cosme D. et al. Each criterion was scored from 0 to 2 (0 = not reported, 1 = limited information, 2 = complete detail). The total score for each study was determined by summing individual criterion scores and normalizing against the maximum possible score (28). Quality assessment was conducted independently by two reviewers and is available in Table 1.

Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). **– FACULTATIVO. APENAS NECESSÁRIO SE FOR FEITA META-ANÁLISE**	Not applicable, as this review didn't include a meta-analysis.
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis. **– FACULTATIVO. APENAS NECESSÁRIO SE FOR FEITA META-ANÁLISE**	Not applicable, as this review didn't include a meta-analysis.
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. **– FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	Not applicable, as this review didn't include a meta-analysis.
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in	Page 8: "The search retrieved 276 articles, 59 from Web of Science, 164 from Scopus, and 53 from PubMed. After removing 77 duplicates, 199 unique articles remained. Screening based on titles and abstracts led to the exclusion of 182 articles that did not meet inclusion criteria. Seventeen full-text articles were assessed for eligibility, of which six were excluded for lacking an experimental model of sepsis or endotoxemia. Ultimately, 11 studies met all inclusion criteria and were included

		the review, with reasons for exclusions at each stage, ideally with a flow diagram. – **MANDATÓRIO**	in the final review. The study selection process is detailed in a PRISMA flow diagram (Figure 1). Key study characteristics and findings are summarized in Table 2 and Table 3, regarding results of animal studies and *in vitro* studies, respectively.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. – **MANDATÓRIO**	Page 9: Table 2 and 3.
Risk of bias within and across studies	19/ 22	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). – **MANDATÓRIO**	Page 10: "Regarding study quality, all included articles achieved a quality score above 75%. However, the criterion with the lowest scores was "evaluation by independent observers, blinding, and evidence of independent repetitions in data collection", suggesting methodological limitations that could introduce bias. This underlines a need for greater rigor in experimental design and validation in future studies exploring the role of the endocannabinoid system in sepsis.
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. – **FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	Not applicable, as this review didn't include a meta-analysis.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of	Not applicable, as this review didn't include a meta-analysis.

		consistency. **– FACULTATIVO. MANDATÓRIO APENAS SE FOR FEITA META-ANÁLISE**	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). **– FACULTATIVO. APLICÁVEL APENAS SE FOR FEITA META-ANÁLISE**	Not applicable, as this review didn't include a meta-analysis.
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). **– MANDATÓRIO**	Page 9: "This systematic review analyzed 11 articles, including both animal and human in vitro models, reflecting the slow progress and limited funding of this subfield. Despite these constraints, some conclusions can be drawn regarding the modulatory role of endocannabinoids in the microvascular pathophysiology of sepsis. This modulation is mediated through various molecular cascades and receptor-specific effects (despite some variability, within the same receptor, by using different activating substances), particularly CB1 and CB2 and vanilloid receptors, discussed individually in this discussion. Additionally, the unique properties of THC, a partial agonist of both CB1 and CB2, warrant specific considerations due to its distinct pharmacological profile. CB1 CB1 is the most well-studied receptor of the endocannabinoid system, with multiple central and peripheral effects, such as in inflammatory conditions and pain modulation. Beyond these well-documented roles, it has been implicated in vascular tone regulation and metabolism, through its presence in endothelial and smooth muscle cells, which, despite expressing lower CB1 levels, retain functional activity [31]. In fact, Wilhelmsen et al. (2014) (Table 3) detected CNR1 (the mRNA encoding CB1R) in various endothelial tissues, providing key experimental validation and supporting its physiological relevance. Notably, this study remains the only one to directly confirm CB1 receptor presence in these cells. As outlined in the results' section, 6 articles investigated the impact of CB1 modulation in septic models (Braile et al. 2020; Toguri et al. 2015; Wilhelmsen et al. 2014; Bányai et al. 2023; Singh et al. 2018; Leite-Avalca et al. 2019). Colectively, these articles indicate a sturdy trend towards beneficial effects of endocannabinoid modulation in the physiological mechanisms of sepsis, especially in the contractile response and blood pressure regulation (Toguri et al., 2015, Singh et al. 2019 and Leite-Avalca et al. 2019). Kianian et al. (2013) proposed a non-CB2 mediated effect on vascular contractility, while Braile et al. (2020) explored CB1's role in angiogenesis and vascular permeability. Addicionally, Leite-Avalca et al. (2019) demonstrated that CB1 receptor antagonism decreased markers of organ dysfunction. However, findings on CB1 receptor expression remain inconsistent, with studies reporting both upregulation and downregulation. These differences may be attributed

			to tissue-specific responses, stage of sepsis at the time of measurement and, most likely, the complex pharmacological behaviour of THC, which was underexplored (Bányai et al. 2023). These variations could significantly impact the interpretation and clinical application of these findings. The results of this review align with findings in different settings, adding a layer of credibility to these results. Similarly to the CB1-mediated hypotensive effects shown by Bányai et al. 2023, Toguri et al. 2015, Singh et al. 2018 (and suggested by Kianian et al. 2013), other studies have proved this effect in hemorrhagic shock [32], after myocardial infarctions [33] and in normal physiological conditions, previously described by Malinowska et al. (2017) [8], addressing the trifasic effect on blood pressure. Despite this, Leite-Avalca et al. (2019) reported a rimonabant-dependent increase in tail temperature, suggesting a vasodilatory effect of CB1 antagonism. Furthermore, Bernardelli et al. (2016) suggests that the vascular responses vary depending on the specific tissue examined, the timing and type of inflammatory insult. These findings imply that CB1's role extends beyond simple hypotensive effects, instead differentially modulating blood vessel contractility. This is clinically significant, as arterial blood pressure regulation remains one of the most complex yet critical aspects of sepsis management, with multiple therapeutic strategies available to the physicians. A compelling finding from this review is CB1's potential role in modulating angiogenesis and vascular permeability, two processes increasingly recognized as possible targets in sepsis. While the role of vascular endothelial growth factor (VEGF) remains debated, its dysregulation can severely impair endothelial function. Insufficient VEGF levels contribute to endothelial apoptosis and reduced survival, while excessive VEGF promotes inflammation, vascular dysfunction and leucocyte adhesion. Braille et al. (2020) demonstrated a partial CB1-mediated effect on VEGF levels, coinciding with ecreased vascular permeability. This observation is noteworthy, as such effect are more commonly attributed to CB2 agonism. [35] These findings highlight the complexity of endocannabinoid signaling in sepsis and underscore the need for further experimental research. CB2 The CB2 receptor is often described as the "shy" counterpart of CB1, sharing 44% overall genomic identity [36] while being much less prevalent in the tissues where CB1 is predominant, the CNS, where it can be upregulated in neuroinflammatory states in glial cells [37]. CB2 is more abundant in immune and lymphoid tissues (e.g., thymus, spleen, and peripheral blood mononuclear cells). Additionally, low levels of CB2 expression have been detected in cardiomyocytes and endothelial cells [24], thereby further broadening the receptor's physiological relevance. However, Wilhelmsen et al. (2014) highlighted a critical issue: when trying to detect CB2 receptor, the evidence was faint and inconsistent. This observation suggests that, under some experimental conditions, CB2's presence is ambiguous, in contrast to earlier studies reporting robust expression. In essence, the reliability of detecting CB2 appears to depend on the specific methods used and further research employing diverse methodological approaches is essential to confirm CB2's expression in endothelial cells and fully unravel its physiological significance. The CB2-related information provided by these articles (4 that studied it individually and another 4 where it is evaluated along other receptor) consistently indicates that stimulation of the CB2 receptor significantly reduces leukocyte adhesion to the endothelial walls. Ramirez et al. (2012) and Rajesh et al. (2007) reported a reduction of the expression in intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), proteins that play a role in leukocyte adhesion and are present in both endothelial and immune cells. In contrast, Braile et al. (2020) reported no measurable effect on adhesion molecules that are exclusively expressed by immune cells (e.g., CD66b and L-selectin). These discrepancies suggest that the anti-adhesive effects of CB2 activation may primarily be mediated via its action on endothelial cells. Despite this, Rom et al. (2013) showed that leukocytes derived from

			CB2 knockout (CB2ko) rats adhered more strongly to the endothelium compared with those from wild-type rats, whereas wild-type leukocytes displayed increased adhesion when interacting with the endothelium of CB2ko rats. Collectively, these observations tend to suggest that CB2 modulates cellular adhesion processes in both immune and endothelial compartments, despite previous conflicting reports. In parallel with these findings, CB2 agonism also enhanced endothelial-barrier function. Ramirez et al. 2012 demonstrated that CB2-dependent mechanisms attenuated LPS-induced endothelial permeability, an effect accompanied by the upregulation of tight-junctions proteins. Similarly, Braile et al. (2020) observed that treatment with the CB2 agonists ACEA and JWH-133 reduced vascular permeability to polymorphonuclear cells; this effect was partially reversible by both CB1 and CB2 antagonists. These findings are particularly relevant in neuroinflammation, where disruption of the blood-brain barrier poses a significant risk to the central nervous system homeostasis. Combined effect of THC in CB1 and CB2 The potential combined effects of tetrahydrocannabinol (THC), a phytocannabinoid extracted from Canabis sativa with CB1 and CB2 partial agonism activity, on both these receptors have also been explored in sepsis-induced circulatory dysfunction. One study (Bányai et al. 2023) reported that THC administration resulted in a reduction of several inflammatory markers, including indices of lipid oxidation, oxidative and nitrative stress, as well as DNA damage , even though it was associated with a reduction in CB1 receptor expression. Moreover, while some studies indicate that THC may induce endothelial-dependent relaxation in thoracic aorta segments, thus suggesting vasodilatory effects, similar end-diastolic volumes and cardiac outputs in LPS-treated groups — regardless of THC administration — suggest that THC does not effectively counteract LPS-induced vasoconstriction. Therefore, the evidence does not conclusively support a vascular benefits of THC in sepsis. Nonetheless, the divergent findings regarding THC's inflammatory effects warrant further investigation, which may be more appropriately addressed in a subsequent, dedicated review, as it does not fall within the scope of this review. Vanilloid receptors Interest in vanilloid receptors has grown markedly in the recent decades. They are responsible for the peripheral transduction of thermal stimuli and nociception, being gated by physical (such as heat, aggression, etc) and chemical stimuli (protons, their more classical agonists capsaicin, menthol, mustard, camphor) and endocannabinoid agonists. TRPV1 has garned particular attention in the endocannabinoid field because of the similarities between endogenous CB1 agonists and TRPV1 agonists (anandamide, for example, is an agonist for both receptors, despite having a greater affinity for the CB1 receptor; this happens with NADA and arachidonyl ethanolamine, in a similar way) [38]. This pharmacological overlap has prompted studies investigating TRPV1's potential role in modulating the endocannabinoid system. Wilhelmsen et al. (2014) reported that administration of NADA and WIN-55,212-2 significantly reduced interleukin-8 (IL-8) levels and decreased the superficial expression of E-selectin, a molecule critical for endothelial leucocytes recruitment that can impair vascular flow and contribute to vascular dysfunction. Importantly, these anti-inflammatory effects were reversed by a TRPV1 antagonist, suggesting that this receptor may confer protection under basal conditions, but adopt a proinflammatory role upon activation. Furthermore, the inhibitory impact of NADA on inflammatory markers was diminished in the presence of CB1 and CB2

			antagonists, implying an interaction between these receptors in regulating inflammation. Future studies that test combined antagonism of CB1, CB2, and TRPV1 on leukocyte adhesion would help clarify the interplay between these receptos, as previous evidence indicates that TRVP1 activation may enhance leukocyte-endothelial adhesion [33]. In contrast, Orliac et al. (2003) examined the contractile effects of anandamide in mesenteric beds and found no significant difference in norepinephrine-induced contraction between septic and control tissues, despite an increase in inducible nitric oxide synthase (iNOS) activity. This observation may indicate limitations in the experimental design or an eveluation at an early time point that failed to capture diminished adrenergic responses — and effect that has been documented in other studies (Mitolo-Chieppa et al. 1996 [40] and Singh et al. 2018). Nevertheless, the study highlights the vasodilatory properties of anandamide, which are potentiated by endotoxemic conditions and modulated via TRPV1. Such findings suggest that modulation of TRPV1-mediated vasodilation may represent a viable therapeutic strategy for addressing septic vascular dysfunction.
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). **– MANDATÓRIO**	Page 13: "Limitations regarding inflammatory models and translational relevance A notable limitation of this review is the heterogeneity of inflammatory models employed across the included studies. Several investigations focused on systemic inflammatory responses that, although partially analogous, do not fully replicate sepsis. For example, two studies examined neuroinflammation and one used alternative inflammatory stimuli (including TNF-alfa-based models) alongise LPS. Moreover, many articles excluded from this review lacked a septic/endotoxemic model where endocannabinoid modulation was assessed, suggesting a potential research gap despite the extensive investigation of endocannabinoids in inflammation. Additionally, only one study (Singh et al. 2018) reported a nonzero mortality rate, casting further doubt on whether a genuine septic state was established in the experimental models. Although yielding intriguing conclusions, its limitations — including the lack of human-based clinical evidence and the variability of experimental models — underscore the need for standardized conditions to better elucidate the complex and sometimes contradictory vascular effects of the endocannabinoid system. Limitations regarding risk of bias assessment Another limitation is the absence of a formal risk-of-bias evaluation. Although tools such as the SYRCLE tool and CAMARADES checklist exist for animal studies, they do not fully reflect typical reporting practices in this field. Notably, the studies included in our review dido not report critical parameters — such as allocation concealment, random housing, or adequate blinding of caregivers and outcome assessors — making it difficult to assess potential biases uring these instruments. We suggest that this omission reflects a misalignment between current risk-of.bias tools and actual reporting standards rather than poor study quality. Nevertheless, for the sake of transparency and comparability, further research should strive to implement and report these methodological safeguards.
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and	Page 14: "This review demonstrates that the endocannabinoid system represents a promising target for sepsis mangement, as its modulation — via CB1, CB2, and TRPV1 receptors — exerts significant effects on key vascular functions, including leukocyte adhesion, vascular tone, endothelial barrier integritu and even

		implications for future research. – **MANDATÓRIO**	angiogenesis. However, the translation of these findings is limited by the heterogeneity of septic and inflammatory models, the scarcity of clinical data, and methodological inconsistencies, highlighting the need for standardized experimental protocols and rigorous bias assessment in future research.
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. – **SEGUIR RECOMENDAÇÕES DA REVISTA**	Page 15: "Funding The authors declare that no funds, grants, or other support were received during preparation of this manuscript. Competing Interests The authors have no relevant financial or non-financial interests to disclose. Authors Contributions All authors contributed to the design and conceptualization of the study. FMVR had a major role in the acquisition, analysis, and interpretation of data, drafting the first draft of the manuscript. EM and MAVC also contributed for the acquisition, analysis, and interpretation of data and reviewed the draft in its posterior versions. All authors read and approved the final manuscript.

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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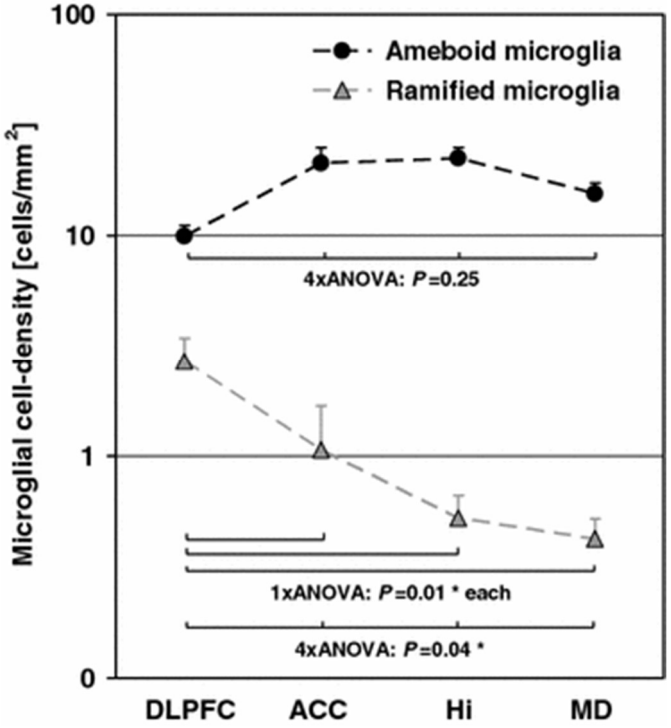

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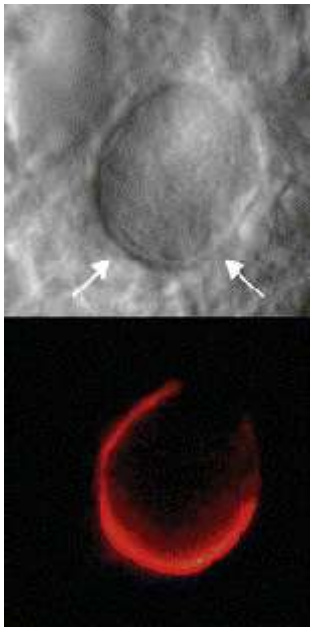

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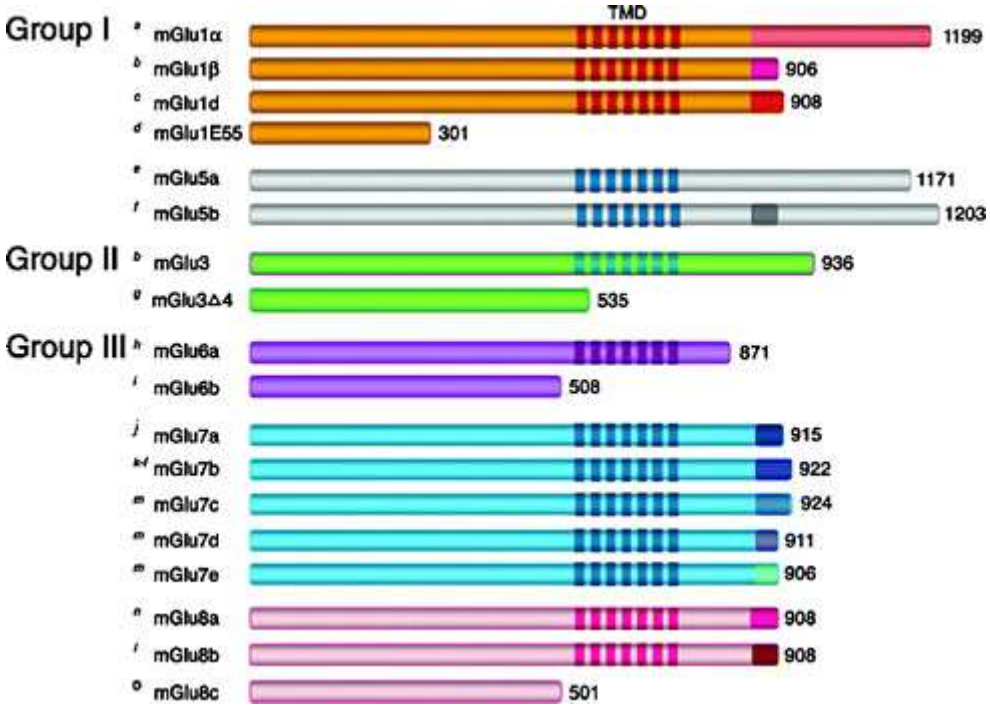

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