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Review

Unveiling the angiogenic effects of cannabinoids: Enhancers or inhibitors?

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ABSTRACT

Cannabinoids are compounds found in the *cannabis sativa* plant. Cannabinoids, such as delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), have potential therapeutic benefits in various medical conditions. Some can activate the cannabinoid receptors type-1 and -2 (CB1 and CB2), that are part of the endocannabinoid system (ECS), alongside the endocannabinoids and their metabolic enzymes. The ECS regulates physiological and cognitive processes and is a potential therapeutic target for a wide range of health conditions like chronic pain, anxiety, and neurodegenerative diseases. Synthetic cannabinoids, are associated with serious health risks, including addiction, psychosis, and death. Nonetheless, some of these molecules are also being explored for pharmacological applications.

Angiogenesis is the process of forming new blood vessels from existing ones, crucial for growth, repair, and tissue maintenance. Dysregulation of this process is associated with several diseases, including cancer, diabetic retinopathy and reproductive pathologies, such as preeclampsia. Recent data suggests that cannabinoids may affect angiogenesis. Here, we reviewed their impact on pro-angiogenic factors, extracellular matrix enzymes and inhibitors, immune-inflammatory responses, angiogenic pathways and functional assays, focusing on the main compounds for each cannabinoid class: THC and CBD for phytocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) for endocannabinoids and WIN-55, JWH-133, XLR-11, LYR-7 and LYR-8, for the synthetic cannabinoids.

Despite conflicting reports about the actions of phytocannabinoids and endocannabinoids on angiogenesis, the ability to modulate the angiogenic process is undoubtedly confirmed. This may open a new therapeutical route for angiogenesis-related pathologies. In addition, synthetic cannabinoids present anti-angiogenic actions in several cell models, hinting their potential as anti-angiogenic drugs.

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, anandamide; Ang, angiopoietin; CAM, chrorioallantoic membrane; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CBD, cannabidiol; CBDV, cannabidivarin; CBG, cannabigerol; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DAGL, diacylglycerol lipase; eCBs, endocannabinoids; ECS, endocannabinoid system; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; FAAH, fatty acid amide hydrolase; FGF, fibroblast growth factor; GPR55, G protein-coupled receptor 55; HBMEC, human brain microvascular endothelial cells; HESC, human endometrial stromal cell; HIF, hypoxia-inducible factors; HUVEC, human umbilical endothelial cells; IFN-γ, interferon-gamma; IL, interleukin; IUGR, intrauterine growth restriction; JAKs, Janus kinases; LPS, lipopolysaccharide; MAGL, monoacylglycerol lipase; Met-F-AEA, 2-methyl-2′-F-anandamide; MMPs, matrix metalloproteinases; mTOR, mammalian target of rapamycin; NAPE-PLD, *N*-acyl phosphatidylethanolamine-specific phospholipase D; PD-1, programmed cell death protein 1; PDGF, platelet-derived growth factor; PECAM-1, platelet endothelial cell adhesion molecule-1; PKB, Protein kinase B; PlGF, placental growth factor; PPARs, peroxisome proliferator-activated receptors; sFLT1, soluble fms-like tyrosine kinase 1; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor-beta; THC, delta-9-tetrahydrocannabinol; THCV, tetrahydrocannabivarin; TIMPs, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cells; TRPV1, transient receptor potential cation channel 1; VEGF, vascular endothelial growth factor.

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1. Introduction

1.1. Cannabinoids

In recent years, cannabinoids, a group of compounds found in the *cannabis sativa* plant, have gained significant attention due to their potential therapeutic benefits in various medical conditions [1]. The most well-known phytocannabinoid is delta-9-tetrahydrocannabinol (THC), responsible for the psychotropic effects of Cannabis [2]. However, recent research has also highlighted the therapeutic potential of non-psychoactive cannabinoids, such as cannabidiol (CBD), which has been shown anti-inflammatory, analgesic, and anxiolytic effects [3]. Nowadays, the interest in minor cannabinoids such as cannabigerol (CBG), cannabidivarin (CBDV) and tetrahydrocannabivarin (THCV) has increased due to their range of therapeutic effects such as antibacterial, anti-inflammatory and neuroprotective, as well as appetite-suppressing and anti-anxiety [4–8]. As consequence, various forms of cannabinoid-based medications have been developed as oral preparations, sprays, and ointments [9]. Despite the growing body of research on cannabinoids, there is still much to be learned about their mechanisms of action, optimal dosing, and potential side effects [10].

The THC molecular targets are the cannabinoid receptor 1 (CB1) and cannabinoid receptor type 2 (CB2) [11,12]. The former is mostly expressed in the central nervous system, while the latter is primarily found in the peripheral nervous system and immune cells. The CBs are G-protein coupled receptors that play a role in several physiological processes.

Besides the phytocannabinoids, there are also endogenous compounds with the ability to activate these receptors, being designated as endocannabinoids. The two best-known endocannabinoids are anandamide (AEA) [13] and 2-arachidonoylglycerol (2-AG) [14]. These compounds modulate several processes, including pain sensation, mood, appetite, immune and reproductive functions [15–18]. However, AEA, like other endogenous mediators, can also activate other receptors besides CBs, such as G protein-coupled receptor 55 (GPR55), transient receptor potential cation channel 1 (TRPV1) and peroxisome proliferator-activated receptors (PPARs) [19].

Although endocannabinoids are primarily produced in response to specific stimuli and are readily available to activate their receptors or be degraded by enzymes, there is currently emerging evidence, suggesting that endocannabinoids can also be stored in cytosolic organelles such as adiposomes [20]. The endocannabinoids AEA and 2-AG, their molecular targets CB1 and CB2, as well as the enzymes that produce and metabolize them, *N*-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and fatty acid amide hydrolase (FAAH) for AEA and diacylglycerol lipase (DAGL) α/β and monoacylglycerol lipase (MAGL) for 2-AG, respectively, [21] constitute the endocannabinoid system (ECS). Given the involvement of the ECS in several physiological functions, it can be a potential therapeutic target for a wide range of conditions, including chronic pain, anxiety and neurodegenerative diseases [19,22,23].

Extensive structure–activity research led to the development of synthetic cannabinoids, a class of compounds that were primarily designed to mimic the effects of phytocannabinoids on cannabinoid receptors but present a higher potency and longer action period. These compounds are marketed primarily as legal alternatives to marijuana and their use can be associated with serious health risks, like addiction, psychosis, and death. XLR-11 is one of the widely used synthetic cannabinoids, which was first synthesized by Pfizer. It is a highly potent CB1 agonist that induces similar effects to those of marijuana, such as euphoria, relaxation, and altered perception [24]. Another synthetic cannabinoid is WIN-55,212-2, which was first produced by researchers at Sterling Winthrop [25]. Like XLR-11, WIN-55 is a potent agonist of CB1 and produces a wide range of effects, including analgesia, sedation, and hypothermia [26]. JWH-133 is a synthetic cannabinoid that has received considerable attention in recent years. Unlike XLR-11 and WIN-55, which are full agonists of CB1, JWH-133 is a selective agonist of CB2. Several studies have shown that JWH-133 has potent anti-inflammatory and analgesic effects and may have potential in the treatment of arthritis and multiple sclerosis [27,28]. LYR-8 and LYR-7 are two other synthetic cannabinoids that are not based on the structure of THC, but, instead, on a novel scaffold that was designed to selectively activate CB2. This receptor activation leads to reduced pro-inflammatory cytokines release, as tumor necrosis factor α (TNF-α) and interleukin (IL)-6, and an increase in anti-inflammatory cytokines, like IL-10. Although little is known about the pharmacological properties of these compounds, initial studies have suggested that they may have potential as analgesic and anti-inflammatory agents [29,30].

1.2. Angiogenesis

Angiogenesis is the process of forming new blood vessels from existing ones and is crucial for growth, repair, and maintenance of tissues. In response to injury, the body stimulates the growth of new blood vessels to the site of the wound, which supports the growth of new tissue and helps the wound healing process [31].

During embryonic development, it is necessary for the formation of the circulatory system and the establishment of blood flow to developing organs [32]. Angiogenesis is also involved in the growth and development of tissues and organs. For example, during puberty, it is essential for bone growth and the increase in body size that occurs during this stage of development [33]. In the female reproductive system, angiogenesis is involved in the growth and maintenance of the endometrial lining, fundamental for successful implantation of the fertilized egg [34,35]. As the placenta develops, angiogenesis is responsible for creating blood vessels, which grow and branch out to form a complex network that surrounds the developing fetus. This network of blood vessels is critical for the proper functioning of the placenta ensuring the healthy fetal growth [36].

1.2.1. Angiogenesis mediators

Angiogenesis is regulated by a complex interplay of growth factors, extracellular matrix components and other signaling molecules [37].

A group of growth factors and cytokines, known as angiogenic factors, stimulate the growth and proliferation of new blood vessels. Some of the key angiogenic factors include the vascular endothelial growth factor (VEGF), produced by a variety of cells. Several members of the VEGF family differ in their molecular structure and biological activity. VEGF-A is the most well-known isoform of VEGF and possesses potent angiogenic activity. VEGF-B is mainly involved in the development of blood vessels in the heart and skeletal muscle, while VEGF-C together with VEGF-D are essential for the development of lymphatic vessels. Additionally, the placental growth factor (PlGF), like VEGF-A, is important for the angiogenic process but also for vascular remodeling. Four isoforms have been identified, from which PlGF-1 and PlGF-2 are the best characterized, whereas the biological relevance of PlGF-3 and PlGF-4 remains to be clarified. There are three main VEGF receptors, known as VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-1 can bind to all VEGF isoforms and PlGF, VEGFR-2 is the primary receptor for VEGF-A, and VEGFR-3 mediates the effects of VEGF-C and VEGF-D on lymphangiogenesis [38]. Additionally, there is a soluble form of the VEGFR1 generated by alternative splicing, the soluble fms-like tyrosine kinase 1 (sFLT1), that prevents the interaction between the ligands and the VEGF receptor, inhibiting the downstream signaling pathways that promote angiogenesis [38]. In normal physiological conditions, sFLT1 acts as a negative feedback mechanism that limits the excessive formation of blood vessels and maintains the delicate balance between angiogenic and anti-angiogenic factors. However, in pathological conditions such as cancer, preeclampsia (PE), and diabetic retinopathy, sFLT1 expression is dysregulated, leading to impaired angiogenesis and tissue ischemia [39]. PlGF binds to VEGFR1, as well as sFLT1 by displacing VEGF-A, allowing more VEGF-A to activate VEGFR-2. VEGFR1

activation by PlGF leads to growth, migration and survival of endothelial cells [40].

Fibroblast Growth Factor (FGF) is another important angiogenic factor that participates in tissue repair, wound healing, and angiogenesis [41]. FGF can bind with high affinity to tyrosine kinase FGF receptors on the surface of endothelial cells, triggering a cascade of signaling events that ultimately lead to the activation of genes involved in cell proliferation, migration and differentiation. FGF also interacts with other pro-angiogenic factors, such as VEGF family members [42].

Platelet-Derived Growth Factor (PDGF) is a potent angiogenic factor produced by platelets. It is involved in wound healing and tissue repair, as well as in the development of various pathological conditions, such as cancer and age-related macular degeneration [43]. Interleukin-8 (IL-8) is a pro-inflammatory cytokine that has been shown to stimulate angiogenesis. This interleukin is produced by a variety of cells, including immune and tumor cells and is part of chronic inflammatory processes, such as rheumatoid arthritis [44].

Angiopoietins are a family of angiogenic growth factors fundamental for the formation and maintenance of blood vessels. However, unlike other angiogenic factors, as VEGF, angiopoietins are not solely pro-angiogenic. Instead, they play a complex role in regulating the balance between angiogenesis and angiostasis, the process of blood vessels shutdown [45]. Among angiopoietin family, angiopoietin (Ang)1 and Ang2 are the most extensively studied members. They have been shown to have opposing effects. Ang1 promotes angiogenesis by activating Tie2, while Ang2 can either promote or inhibit the process depending on the context in which it is expressed.

CD31, also known as platelet endothelial cell adhesion molecule-1 (PECAM-1), is a transmembrane glycoprotein that is predominantly expressed on the surface of endothelial cells, platelets, and leukocytes. It is a member of the immunoglobulin superfamily of adhesion molecules intervening in cell adhesion, migration, and signaling [46]. CD31-mediated cell–cell adhesion is important for the formation and maintenance of endothelial cell junctions, which are critical for the integrity and function of the vascular endothelium [47].

Hypoxia-inducible factors (HIFs) are transcription factors that are involved in the cellular response to hypoxia, or low oxygen levels. HIFs are composed of two subunits: an oxygen-sensitive alpha subunit (HIF-α) and a constitutively expressed beta subunit (HIF-β). In normoxic conditions, HIF-α is rapidly degraded by the proteasome, but in hypoxic conditions, HIF-α stabilizes and translocates to the nucleus where it forms a heterodimer with HIF-β and activates the transcription of target genes. Dysregulation of this factor has been linked to many types of cancer, wherein the hypoxia of the tumor causes cellular mechanisms that promote adaptation and survival, including angiogenesis, metastasis, and treatment resistance [48].

Matrix metalloproteinases (MMPs) are involved in extracellular matrix degradation, an important step during the first phases of the angiogenesis process, to facilitate endothelial migration and remodeling of the endothelial cell microenvironment [49]. Tissue inhibitors of metalloproteinases (TIMPs), on the other hand, are endogenous inhibitors of MMPs that bind to the catalytic domain of MMPs in a 1:1 stoichiometric manner and act as regulators of MMPs preventing their activation [50]. There are four TIMPs isoforms (TIMP-1 to TIMP-4) that can inhibit various MMPs, but each TIMP isoform has a different inhibitory profile. TIMP-1 and TIMP-2 can inhibit MMP-1, MMP-2, MMP-3, and MMP-9, but present little or no inhibitory activity against MMP-7 and MMP-13. In contrast, TIMP-3 has a broad inhibitory activity against MMP-2, MMP-3, MMP-7, MMP-9, and MMP-14, though without inhibitory activity against MMP-1 and MMP-8. TIMP-4 has a similar profile as TIMP-1 and TIMP-2 against MMP-2 and MMP-9 but has a higher affinity for MMP-14 and can inhibit the activation of pro-MMP-2 by MMP-14. Nevertheless, all are important for maintaining the balance between MMPs activity and ECM turnover, and dysregulation of TIMP expression or function has been associated with various pathological conditions [51].

1.2.2. Angiogenesis and immune cells

The immune cells are involved in the mechanisms of endothelial cell proliferation and migration and can contribute to the angiogenic process either directly by producing angiogenic growth factors or indirectly by secreting cytokines and other mediators able to modulate cell–cell interactions. Immune cells can either stimulate or inhibit angiogenesis depending on subset specificity and activation status. Tissue resident cells (e.g., mast cells, macrophages), circulating immune cells (e.g., neutrophils, monocytes, eosinophils) and other immune cells serve as both sources and targets of angiogenic molecules [52]. Inflammation-associated angiogenesis is a dynamic reaction that can alter the process of inflammatory tissue repair and resolution. Inflammatory processes often precede angiogenesis, leading to the recruitment of immune cells to the site of injury or infection, thus stimulating the formation of new blood vessels. Among the various cells involved, macrophages play a crucial role in angiogenesis. Macrophages can adopt different subsets with distinct functions. M2 macrophages have a tendency to promote angiogenesis by releasing factors, such as VEGF and MMPs that promote blood vessel formation [53]. Conversely, M1 macrophages exhibit anti-angiogenic properties and release factors like thrombospondin-1 that inhibit the formation of blood vessels [54]. Similarly, regulatory T cells (Tregs), defined as CD4 + CD25 + forkhead box P3 (FoxP3) + T cells, that are typically associated with immune response suppression, also have the ability to modulate angiogenesis [55]. The mechanisms by which Tregs suppress or promote the immune system are complex but mostly involves the production of immunosuppressive cytokines such as IL-10, IL-35, and transforming growth factor (TGF-β), as well as direct cell-to-cell interactions and cytolysis mediated by granzyme A and B.

Interactions between immune cells and the vascular system are also involved in cancer in addition to inflammatory diseases. Another important aspect to consider is the role of immune checkpoints, such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). These molecules are involved in immune regulation and can affect angiogenesis. In cancer immunotherapy, specific antibodies can be used to block these immune checkpoints, leading to enhanced anti-tumor immune responses. This blockade can indirectly influence angiogenesis within the tumor microenvironment [56].

1.3. Angiogenesis-related pathologies

Excessive angiogenesis is a hallmark of several pathologies, being often a key factor in the growth and progression of cancer. Some angiogenic factors are produced by tumor and inflammatory cells, and is an important factor in the development of many pathological conditions, such as cancer and chronic inflammatory diseases [57].

Tumor cells produce angiogenic factors that stimulate the growth of new blood vessels to the site of the tumor, essential for the necessary oxygen and nutrients for tumor cells grow and proliferation [58]. Angiogenesis also provides a route for cancer cells to metastasize or spread to other parts of the body. Inhibition of angiogenesis has become a promising strategy and several anti-angiogenic drugs have been developed and are currently being used in cancer treatment to target the tumor's blood supply and slow its growth [59].

Chronic inflammation is characterized by the persistent activation of the immune system, which can lead to the appearance of new blood vessels. This can contribute to the progression of the underlying disease, as well as to the development of secondary complications, such as tissue fibrosis [60]. Some chronic inflammatory diseases also present excessive angiogenesis. For example, rheumatoid arthritis progression is also associated to the growth of new blood vessels to the site of inflammation, contributing to its persistence and joint damage [61]. Additionally, age-related macular degeneration is a common eye condition characterized by the abnormal growth of new blood vessels in the retina, causing vision loss [62].

Retinopathy is a disorder that impacts the retina, which is the light-sensitive tissue located at the back of the eye. Although there are various

types of retinopathy, one of the prevalent forms is diabetic retinopathy. In this disease, the small blood vessels in the retina become damaged and leaky, leading to reduced supply of oxygen and nutrient to the retinal cells [63]. Consequently, growth factors are released to stimulate the formation of new blood vessels. This can result in vision loss or blindness if left untreated [63].

One of the placental pathologies associated with impaired angiogenesis is PE, a pregnancy-related disorder characterized by high blood pressure and proteinuria. In PE, abnormal angiogenesis leads to inadequate blood flow and oxygen supply to the placenta, resulting in placental ischemia and maternal symptoms, like high blood pressure and proteinuria and fetal distress [64]. Intrauterine growth restriction (IUGR) is another placental pathology associated with impaired angiogenesis in the placenta, insufficient blood flow and oxygen supply to the fetus [39]. In addition to PE and IUGR, angiogenesis is also involved in the development of other placental disorders, such as placental abruption, placenta previa, and placental insufficiency. These conditions are characterized by abnormal blood flow in the placenta, which can lead to fetal growth retardation, premature birth, and other pregnancy-related complications [65,66].

1.4. Cannabinoids and immune cells

The immune system exerts a crucial influence on the regulation of angiogenesis by carefully balancing the interplay between pro-angiogenic and anti-angiogenic factors. Disruptions caused by cannabinoids can potentially disturb this delicate equilibrium, thereby influencing the intricate processes of angiogenesis.

The ECS possesses immunomodulatory properties. CB1 and CB2 are expressed in several immune cells and cannabinoids modulate immune cells interactions and alter cytokine production [67–69]. The ECS modulates the inflammatory responses of macrophages. AEA, 2-AG as well as the specific CB2 agonist JWH-133 efficiently decrease the inflammatory response of M1 macrophages by decreasing the secreted IL-6, IL-8, TNF-α, TGF-β and VEGF. being CB2 activation of particular importance [70].

Human lung macrophages possess cannabinoid receptors and have the capability to produce endocannabinoids. When these receptors are activated by specific synthetic agonists such as JWH-133, they significantly inhibit the production of VEGF-A, VEGF-C, and angiopoietins and exert a moderate influence on IL-6 secretion [71]. Similar effects have also been observed in neutrophils as JWH-133 is able to modulate several aspects of neutrophil-assisted angiogenesis mediated by the production of VEGF-A [72].

The anti-inflammatory effects CBD on activated macrophages has also been reported. This phytocannabinoid induces a cytotoxic effect on LPS-activated THP-1 monocytes derived macrophages and reduces the pro-inflammatory markers TNF-α IL-1β and IL-6 as well as suppressing VEGF expression [73]. In addition, it attenuated the production of IL-6 and TNF- α in LPS-stimulated RAW264.7 macrophages [74].

Emerging evidence concerning the interaction between cannabinoids and immune checkpoints strongly suggests that cannabinoids possess the ability to disrupt the functionality of T cells that specifically target tumors by directly engaging the CB2 receptor [75]. These findings suggest that caution should be exercised when using cannabinoid-derived drugs alongside immunotherapy, as the ECS plays a role in suppressing T-cell immunity against cancer. Conversely, cannabinoids have demonstrated protective and anti-inflammatory effects *in vivo* by contributing to the development of functional FOXP3 + Tregs during lipopolysaccharide (LPS)-induced sepsis [76]. In addition, the impact of cannabinoids on the tumor microenvironment remains a topic of debate. For instance, the use of THC and AEA has been shown to diminish the therapeutic benefits of PD-1 blockade [77]. Furthermore, elevated levels of AEA in the bloodstream have been associated with lower overall survival rates in cancer patients [78].These observations present new possibilities for cannabinoid-based interventions in diverse inflammatory and immune-related diseases, offering potential avenues for future research and treatments.

1.5. Cannabinoids and pro-angiogenic factors

The phytocannabinoid responsible for the psychotropic effects of cannabis, THC, increases VEGF-A protein expression in colorectal cancer cell lines [79]. However, on Wistar rats treated with 3 mg/kg, it decreases offspring blood vessel density, together with a decrease in VEGF and VEGFR2 protein expression [80]. On the other hand, the other main phytocannabinoid, CBD, has been shown to decrease vascular structure, vessel size and VEGF protein expression in xenografts of human lung cancer in a dosage of 6.4 mg/day [81]. Additionally, a reduction on VEGF mRNA was observed on mice with ovarian hyperstimulation syndrome when a dosage of 30 mg/kg was applied [82]. An opposite effect was observed in human umbilical endothelial cells (HUVEC) and Sprague Dawley rats, since CBD alginate gel produced an increase in VEGF gene [83]. Although the phytocannabinoids show the ability to modulate angiogenic factors, their actual effect is not consensual, probably due to the use of different dosages and model of study.

As for the endocannabinoids, AEA induced a decrease in VEGF-C protein levels in xenografts of HCT116 colon cancer cell line in zebra fish [84]. In addition, in HTR8/SVneo, can increase endothelial-like structure in the tube formation assay, VEGF, VEGFR, sFLT1 and PlGF gene expression via CB1 and CB2 activation [85]. In the same cell line, the other major endocannabinoid, 2-AG, increases PlGF transcription through CB1 and CB2 [85]. However, in HUVEC treated with a MAGL inhibitor to increase 2-AG levels, a down regulation of VEGF and FGF-2 proteins was verified [86]. Another endocannabinoid-like mediator, *N*-arachidonoylserine was found to increase VEGF-C, VEFR2 and VEGFR3 protein production in primary human dermal microvascular endothelial cells, being these effects partially inhibited through the knockdown of the G-protein coupled receptor GPR55 [87]. Despite these two reports, there is still a lot of room to explore the role of 2-AG on angiogenesis, in order to have a clearer view of its effects.

The ability of synthetic cannabinoids to modulate the pro-angiogenic factors has been demonstrated in various studies. For instance, the treatment of epidermal tumor cell line PDV C57B xenografts with WIN-55,212–2 led to a decrease in the protein levels of VEGF, PlGF, Ang-2 and EGF receptor [88]. Moreover, LYR-8 was found to reduce VEGF gene expression in HT-29 colon cancer cells [89], while, alongside, LYR-7, was able to reduce VEGF induced angiogenesis [30].

In pregnant cannabis consumers, the endothelial marker CD31 protein expression is decreased in the placenta [90]. However, in the colorectal cancer cell lines HCT116, SW480, RKO and HT-29, an opposite effect was verified, as THC increased CD31 expression [79]. Also, CBD has been shown to increase CD31 protein expression in HUVEC and Sprague Dawley rats [83]. Synthetic cannabinoids, such as WIN-55,212-2 reduce CD31 expression levels in endometriosis mouse model in a dosage of 1 mg/kg [91], as well as in xenografts of MDA-MB231 breast cancer cells in CB-17 [92]. Moreover, in the latter study, JWH-133 also has the ability to decrease CD31 expression [92]. Seemingly, while phyto- and endocannabinoids increase this endothelial marker expression, the synthetic cannabinoids tend to decrease it. Thus, these molecular studies *in vitro* and *in vivo* demonstrate that cannabinoids exert effects through various angiogenic mechanisms, suggesting that they may have great potential as new antiangiogenic drugs (Fig. 1).

1.6. Cannabinoids and MMPs

MMPs and TIMPs play a crucial role in angiogenesis, as they regulate the proteolysis of the extracellular matrix and the release of pro-angiogenic factors, such as VEGF, FGF and PDGF, which are essential for the formation of new blood vessels (Fig. 2).

In A549 lung carcinoma and HUVEC cell lines, THC induced a decrease in cell migration and an increase in TIMP-1 [93]. Furthermore,

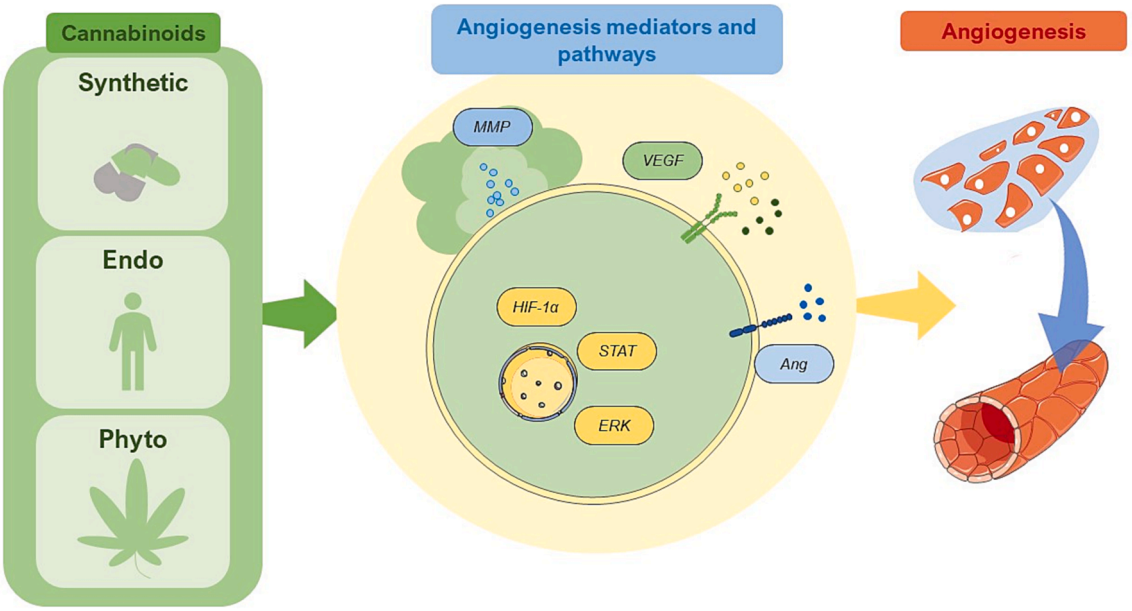

Fig. 1. Summary of the main transcription factors and angiogenesis mediators known to be influenced by phyto, endo and synthetic cannabinoids during the angiogenic process. Several studies have focused their research on the impact of cannabinoids in the VEGF family, Ang and MMP/TIMP families. Additionally, HIF-1α, STAT and ERK have been the main transcription factors linked to the angiogenesis process, where the effects of cannabinoids have been evaluated.

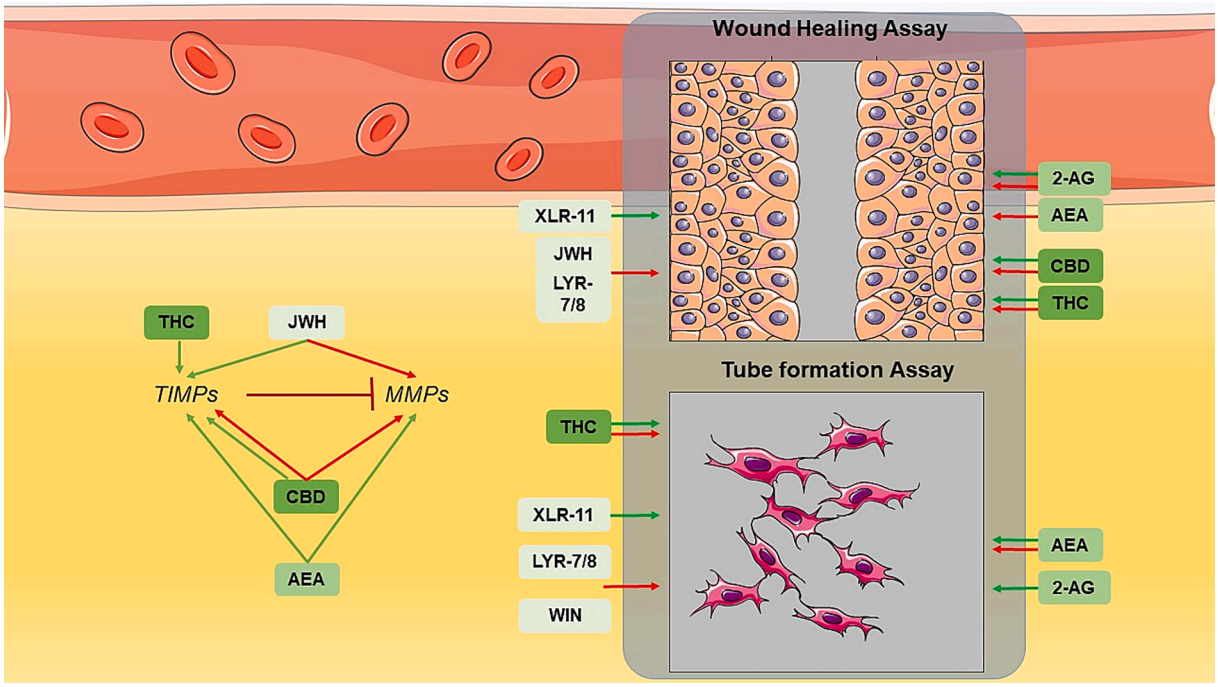

Fig. 2. Effects of cannabinoids on MMPs, TIMPs and tube formation and wound healing assay. Red arrows depict decrease in expression, transcription or activity on MMPs, while on the functional assays it represents decrease in cell migration or tube formation. Green arrows depict an increase in expression, transcription or activity on MMPs, while on the functional assays it represents an increase in cell migration or tube formation. Both arrows in the same cannabinoid represent contrary results in different studies.

in keratinocytes isolated from psoriatic patients, CBD decreased MMP-1/2/3/7 and TIMP-1/2 [94]. Also, CBD decreased MMP-9 and TIMP-1 expression in U87-MG and T98G glioma cells [95]. Similar observations were reported in HUVEC cells where CBD in higher doses reduced MMP-9 and TIMP-1 expression [96]. On the contrary, in HTR8/SVneo cells, AEA is able to increase MMPs and TIMPs transcription [85], while the stabilized version of AEA, 2-methyl-2′-F-anandamide (Met-F-AEA) produced a reduction of MMP-2 activity on endothelial cells [97]. JWH-133 has also shown the ability to cause an increase in TIMP-1 in A549 lung carcinoma and HUVEC [93], as well as a decrease in MMP-2 [98]. All this data demonstrated that cannabinoids affect MMP and TIMP molecules, key factors in cell motility, invasion and cell proliferation, crucial processes for angiogenesis.

1.7. Cannabinoids, angiogenic pathways and transcription factors

Cannabinoids have been found to modulate angiogenesis, by regulating various molecular pathways, which may have potential therapeutic implications for cancer and other diseases characterized by abnormal blood vessel growth. Among the extensively studied angiogenic pathways, activated signal transducer and activator of transcription (STAT), extracellular signal-regulated kinase (ERK), and Rho/GTPase stand out. Protein kinase B (PKB, or Akt) promotes angiogenesis by activating several downstream effectors, including endothelial nitric oxide synthase (eNOS) and mammalian target of rapamycin (mTOR) through phosphorylation [99]. The ERK pathway is also activated by growth factors but is primarily involved in promoting cell proliferation and survival. ERK is activated downstream receptor tyrosine kinases by the small GTPase Ras, which activates the MAP kinase cascade. Activated ERK promotes angiogenesis by inducing the expression of several angiogenic factors, including VEGF, and promoting the survival and proliferation of endothelial cells [100]. Fig. 3 visually presents a comprehensive depiction of the influence of cannabinoids on various pro-angiogenic factors, pathways, and transcription factors. The STAT pathway is activated by cytokines, such as IL-6, that bind to membrane receptors of endothelial cells. This leads to the activation of Janus kinases (JAKs), which phosphorylate and activate STATs that induce the expression of several angiogenic factors, leading to the survival and proliferation of endothelial cells [101].The Rho family of GTPases, which includes RhoA, Rac1, and Cdc42, plays a key role in regulating angiogenesis, or formation of new blood vessels from pre-existing ones. RhoA is the most well-studied member of the Rho family in angiogenesis. RhoA activation is required for endothelial cell proliferation, migration, and tube formation in response to pro-angiogenic signals, like VEGF and FGF [49]. In addition, it promotes the formation of focal adhesions and stress fibers, necessary for endothelial cell migration and tube formation.

Cannabinoids may affect the different angiogenic pathways. It was reported that THC decreases Rho/GTP in HUVEC [90]. A similar result was observed in human placenta of pregnant cannabis consumers [90]. In addition, a reduction in STAT3 phosphorylation was observed on HTR8/SVneo extravillous trophoblast cells with a higher dose of THC [102]. Moreover, this phytocannabinoid increases STAT1 nuclear translocation and promoter binding to induce angiogenesis-related genes transcription [79]. On the other hand, CBD decreases invasiveness, AKT and ERK activation, as well as HIF-1α expression in U87-MG and T98G glioma cells [95]. Similar observations were obtained on MCF7 breast cancer cells regarding the ability of CBD to downregulate HIF-1α [103]. Met-F-AEA, has also been shown to decrease ERK phosphorylation in endothelial cells [97].

AEA and 2-AG, caused similar results. In HTR8/SVneo, these endocannabinoids decrease HIF-1α transcription levels, through the involvement of STAT3 activation and a reduction of AKT phosphorylation via CB1 and CB2 activation [85]. WIN-55,212-2 can also decrease ATF2, ERK1/2, p38, MSK1 and JNK activation in the immortalized endometriotic cell line (12Z) and in the human endometrial stromal cells (HESC) [91]. In turn, chronic hypoxia suppressed the expression of the genes encoding cannabinoid receptors [104]. Although additional research is required to clarify the impact of hypoxia-mediated endocannabinoid inhibition on both healthy and cancer cells, the observed suppression of cannabinoid receptors was proposed to be linked to cancer progression.

1.8. Cannabinoids and angiogenic functional assays

The most widely functional assays used to mimic angiogenic processes include the wound healing assay to evaluate cell migration, the tube formation assay, which assesses the formation of blood vessel-like structures in an appropriate extracellular matrix support, as well as the chrorioallantoic membrane (CAM) assay that uses the chick embryo chrorioallantoic membrane to study the impact of drugs in the development of blood vessels. Several studies have explored the effect of cannabinoids and obtained controversial results, although all have demonstrated that these compounds impact cell migration and tube formation. Table 1, 2, and 3 present comprehensive information regarding the significant findings concerning the impact of

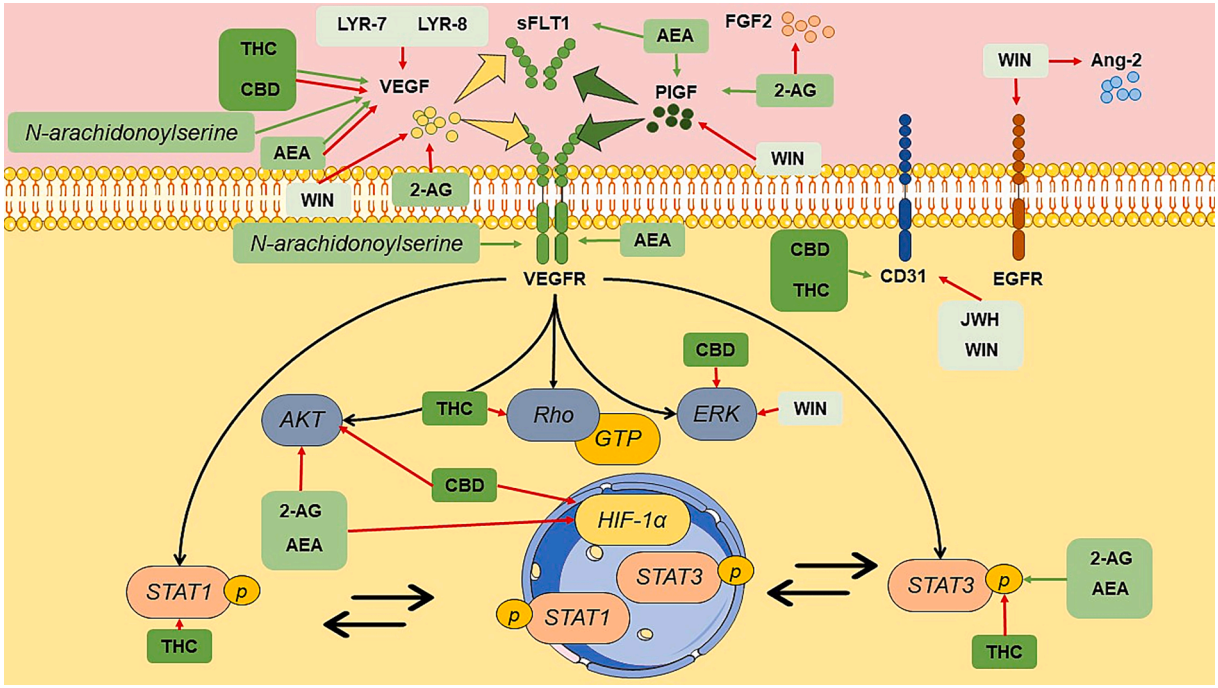

Fig. 3. Effects of cannabinoids on pro-angiogenic factors, pathways and transcription factors. Red arrows depict downregulation or decrease in expression or transcription. Green arrows depict upregulation or increase in expression or transcription. Both arrows in the same cannabinoid represent contrary results in different studies.

Table 1
Effects of phytocannabinoids on angiogenesis.

Cannabinoid	Model	Concentration	Effect	Reference
THC	HUVEC cell line	10 μM	Decreased tube length, GTP-Rho, pMLC	[90]
	Rhesus monkey placenta	2.5 mg/kg/day	Reduced placental blood flow, microinfartations, decreased angiogenesis and vasculature development genes	[105]
	Colorectal cancer cell lines HCT116, SW480, RKO, and HT-29 and mice xenograph	5 μM	Upregulation of CD31 and VEGF. Increased migration, tube formation and wound healing.Increased VEGFA. STAT1 translocation and promoter region binding.	[79]
	Wistar rats	3 mg/kg	Ovaries of rats offspings with reduced blood vessel density in association with decreased expression of the pro-angiogenic factor VEGF and its receptor VEGFR-2.	[80]
	A549 lung carcinoma cells, HUVEC	3 μM	Decreased cell migration, increased TIMP-1	[93]
	HTR8/SVneo, BeWo	15 μM	Decreased migration, invasion and STAT3 phosphorylation in HTR8	[102]
CBD	Mice with ovarian hyperstimulation syndrome	30 mg/kg	Decreased VEGF mRNA with CBD treatment	[82]
	HUVECs, mouse embryonic fibroblast cells of NIH 3 T3	CBD alginate hydrogel	Increased VEGF and EGFL6 transcription	[83]
	Sprague Dawley rats	N/D	Increased wound healing, CD31	[83]
	Keratinocytes isolated from psoriatic patients	4 μM	Decrease in MMP-1/2/3/7, TIMP	[94]
	Human lung cancer cells, NCI-H1437 xenograph	6.4 mg/daythrough inhalation	Reduction in the vascular structure and size of vessels in CBD-treated tumors, decreased VEGF	[81]
	A549 lung carcinoma cells, HUVEC	3 μM	Decreased cell migration, increased TIMP-1	[93]
	U87-MG and T98G glioma cells	05–12 μM	Decreased invasiveness, AKT, ERK, HIF-1α, decreased MMP-9, TIMP-1, TIMP-4, VEGF	[95]
	HUVEC	5–12 μM	Inhibited cell migration, invasion and sprouting, decreased MMP-9, TIMP-1	[96]
	MCF7	2 μM	Downregulation of HIF-1α	[103]
Cannabis consumers	Human placenta	N/D	Decreased CD31, GTP-Rho, pMLC	[90]

N/D non determined.

Table 2
Effects of endocannabinoids on angiogenesis.

Cannabinoid	Model	Concentration	Effect	Reference
AEA	HCT116 colon cancer cell line xenograph in zebrafish	10 nM	Decreased VEGF-C protein levels	[84]
	HTR8/SVneo	10 μM	Increased tube formation, VEGF, VEGFR, MMP, TIMP, VEGFR, PlGF. Increase p-STAT3 and decrease p-AKT and HIF-1α	[85]
	HUVEC, lung endothelial cells	10 μM	Decreased tube length and branching points. Decreased wound healing closure	[107]
	Sprague-Dawley rats	3 mg/kg/day	Decrease in pup brain ET_B receptor	[109]
	endothelial colony-forming cells	1 μM	Promoted tube formation through TRPV1	[106]
	HUVEC cells	FAAH inhibitor URB597	Decreased tube formation and wound healing	[107]
	Endothelial cells	2-methyl-2′-F-anandamide (Met-F-AEA)	Decreases capillary formation, ERK phosphorylation, MMP2 and CAM assay	[97]
2-AG	HTR8/SVneo	10 μM	Increased wound healing, tube formation, PlGF transcription. Increase p-STAT3 and decrease p-AKT and HIF-1α	[85]
	HUVEC	MAGL inhibitor URB602	down-regulation of VEGF and FGF-2, reduction in the number of vessels. Reduced migration	[86]
***N*-arachidonoyl serine**	Primary human dermal microvascular endothelial cells (HMVEC)	0.1–10 μM	Increased migration, tube formation, wound healing, CAM assay, VEGF-C, VEGFR2, VEGFR3	[87]

phytocannabinoids, endocannabinoids, and synthetic cannabinoids respectively, on the process of angiogenesis.

THC was able to decrease tube formation in HUVEC [90]. In addition, a reduction in migration and invasion was observed on HTR8/SVneo with a higher dose of THC, dependent of CB1 and CB2 [102]. Moreover, in rhesus monkey placenta, a reduced placental flow, microinfarcts, together with decreased angiogenesis and vasculature development, were reported with a dose of 2.5 mg/kg/day [105]. Contrarily, effects on cell migration were also reported in the colorectal cancer cell lines HCT116, SW480, RKO and HT-29, as well as in mice xenograft, where THC increased cell migration, tube formation and wound healing [79]. CBD has also been shown to decrease vascular structure and vessel size in xenografts of human lung cancer in a dosage of 6.4 mg/day [81]. However, an opposite angiogenic effect was observed in HUVEC and Sprague Dawley rats, since CBD alginate gel produced an increase in wound healing [83]. Furthermore, in keratinocytes isolated from psoriasis patients, CBD decreased cell migration [93]. Similar observations were reported in HUVEC where CBD in higher doses reduced cell migration, invasion and sprouting [96].

In addition, AEA, in HTR8/SVneo, was able to increase tube formation [85]. A similar effect was also reported with lower doses in endothelial colony-forming cells through TRPV1 [106]. However, in HUVEC

Table 3
Effects of synthetic cannabinoids on angiogenesis.

Cannabinoid	Model	Concentration	Effect	Reference
XLR-11	Human brain microvascular endothelial cells (HBMECs)	0.001–1 μM	Increased migration rate and tube formation	[108]
WIN 55,212-2	Endometriosis mouse model	1 mg/kg intraperitoneal	Reduction of K67, CD31	[91]
	endometriotic epithelial (12Z) and endometrial stromal (HESC) cell lines	1–50 μM	Decreased ATF2, Erk1/2, p38, MSK1, JNK	[91]
	HUVECs	1–50 μM	Tube formation disruption	[91]
	xenografts of the epidermal tumour cell line PDV C57B	6,7 μg/μL on a flux of 0.52 μL/h	Decreased VEGF, PlGF, Ang-2, EGF receptor	[88]
	xenografts of MDA-MB231 breast cancer cells in CB-17 mice	5 mg/kg/d intraperitoneal	Decreased CD31	[92]
	MG-63 human osteosarcoma cell line, HUVECs	20 μM	Decreased invasion	[110]
JWH-133	xenografts of the epidermal tumour cell line PDV C57B	6,7 μg/μL on a flux of 0.52 μL/h	Decreased VEGF, PlGF, Ang-2, EGF receptor	[88]
	xenografts of MDA-MB231 breast cancer cells in CB-17 mice	5 mg/kg/dintraperitoneal	Decreased CD31	[92]
	A549 lung carcinoma cells, HUVEC	3 μM	Decreased cell migration, increased TIMP-1	[93]
	A549 non-small lung cancer cells, HUVEC	1–100 μM	Decreased endothelial cell migration and MMP-2 secretion	[98]
LYR-7	HUVEC	1–20 μM	Decreased tube formation, wound healing and migration and VEGF-induced angiogenesis	[30]
LYR-8	HT-29 human colon cancer cells, xenografted tumor tissues	10 μM	Reduced HIF-1α and VEGF	[89]
	HUVEC	1–20 μM	Decreased tube formation, wound healing and migration and VEGF-induced angiogenesis	[30]

cells and lung endothelial cells, an opposite effect on tube formation, as well as a decrease in branching points and wound healing closure was described with the same AEA concentration [107]. Moreover, URB, a FAAH inhibitor to increase AEA levels, reduced tube formation and wound closure in HUVEC [107]. 2-AG has also the ability to increase wound healing and tube formation [85]. However, in HUVEC treated with a MAGL inhibitor to increase 2-AG levels, a reduction on the number of vessels and cell migration was verified [86].

It has been shown that XLR-11 is able to increase migration and tube formation in human brain endothelial cells [108]. A contrary result was observed with JWH-133 in A549 lung carcinoma and HUVEC, which caused a decrease in cell migration [93]. Additionally, LYR-8, as well as LYR-7 were able, in HUVEC, to reduce tube formation, wound healing and cell migration [30]. A disruption in tube formation was also reported with WIN-55,212–2 in HUVEC [91].

2. Conclusion

Over the past 20–30 years, significant progress has been made in cannabinoid research, including the discovery of the ECS and an increasing number of related mediators, precursors, and enzymes. Given the involvement of this system in several physiological functions, its potential as a pharmacological target for many pathologies has been explored.

Angiogenesis, the process of blood vessel formation, is involved in physiological processes, such as placentation, endometrial remodeling, wound healing, and growth. Unfortunately, its impairment is also associated with several pathologies, such as cancer and PE among others. Nevertheless, the study of the role of cannabinoids in the angiogenic processes is in its early days. Given the increase in cannabis consumption and cannabinoid-based drugs, caution is required, since the described studies show the ability of the main phytocannabinoids to disrupt angiogenesis. Additionally, changes in the angiogenic process caused by the endocannabinoids hint a possible role in the genesis of many angiogenesis-related pathologies and the ECS as a target for their treatment. Although all types of cannabinoids have demonstrated the ability to induce or inhibit angiogenesis, it appears that synthetic cannabinoids consistently inhibit the angiogenic process. This suggests a potential role in treating angiogenesis-related pathologies, including cancer. Nevertheless, there is still a lot to be explored given the low number of studies involving endocannabinoids.

CRediT authorship contribution statement

J. Maia: Writing – original draft. **B.M. Fonseca:** Writing – review & editing. **N. Teixeira:** Writing – review & editing. **G. Correia-da-Silva:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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