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REVIEW

Dengue and endothelium: a perspective on the pathophysiological implications of endothelial activation in dengue and its therapeutic possibilities

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Abstract

Introduction: Dengue is a widely spread viral febrile illness, with nearly half of the world's population, approximately four billion people, living in areas at risk of dengue infection. It is often the leading cause of illness in these high-risk areas. Dengue is caused by four virus serotypes (Dengue 1, Dengue 2, Dengue 3, and Dengue 4). This virus is transmitted through the bites of infected female mosquitoes, primarily *Aedes aegypti* and *Aedes albopictus*. There is no specific medication to treat dengue, the and current management of the disease is symptomatic. Thus, an urgent search for specific therapeutic alternatives targeting the virus or the pathophysiological mechanisms responsible for severe forms of the disease is critical. The aim was **t**o gather experimental evidence on the significance of endothelial activation in the pathophysiology of dengue, as available in the scientific literature.
Materials and methods: A literature search were conducted in English using the PubMed, Scopus, and ScienceDirect databases with the keywords dengue, endothelium, pathophysiology, and endothelial activation.
Results: Experimental evidence has shown that endothelial dysfunction is central to the clinical manifestations of severe dengue.
Discussion: The identification of pathophysiological mechanisms at the endothelial cell level has revealed numerous therapeutic targets for dengue, which need to be validated through clinical studies.

Keywords: Dengue, Endothelium, Cytokine, Immunity, Endothelial Dysfunction, Vascular Leakage.

Dengue y endotelio: una mirada desde las implicaciones fisiopatológicas de la activación endotelial en el dengue, hasta sus posibilidades terapéuticas

Resumen

Introducción: El dengue es una enfermedad viral febril muy difundida en el mundo; casi la mitad de la población mundial, alrededor de cuatro billones de personas, vive en áreas con riesgo de dengue; y es a menudo una causa principal de enfermedad en las áreas con riesgo. El dengue es causado por un conjunto de cuatro serotipos de virus con el mismo nombre (Dengue 1, Dengue 2, Dengue 3 y Dengue 4). Este virus es transmitido por la picadura de la hembra infectada de los mosquitos *Aedes aegypti* y *Aedes albopictus*. No existe un medicamento específico para tratar el dengue, el manejo de la enfermedad es hasta la fecha sintomático, por lo que es urgente la búsqueda de alternativas terapéuticas específicas para el virus o para los mecanismos fisiopatológicos responsables de las formas graves de esta enfermedad. El objetivo de este estudio fue recopilar las evidencias experimentales significativas de la importancia de la activación endotelial en la fisiopatología del dengue, disponibles en la literatura científica.
Materiales y métodos: Se realizó una búsqueda de literatura en inglés en las bases de datos Pubmed, Scopus y ScienceDirect, con las palabras claves: Dengue, endotelio, fisiopatología, activación endotelial.
Resultados: Las evidencias experimentales han permitido demostrar que la disfunción endotelial es el núcleo de las manifestaciones clínicas de las formas de dengue grave.
Discusión: El establecimiento de mecanismos fisiopatológicos a nivel de la célula endotelial, han permitido evidenciar numerosos blancos terapéuticos para el dengue, que es necesario empezar a validar con estudios clínicos.

Palabras claves: Dengue; Endotelio; Citocina; Inmunidad; Disfunción endotelial; Fuga vascular

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Introduction

Dengue is an arboviral disease caused by the dengue virus (DENV 1-4), which is transmitted through the bite of infected female mosquitoes of the *Aedes aegypti* and *Aedes albopictus* species. Dengue is hyperendemic in tropical and subtropical climates worldwide, primarily in urban and semi-urban areas[1]. The dengue virus has a relatively recent evolutionary history and includes four serotypes that originated approximately 1000 years ago. It is a relatively new pathogen, and it is believed that person-to-person transmission mediated by *Aedes aegypti* mosquitoes began approximately three centuries ago. The establishment of an urban cycle for the vector is thought to have been preceded by independent events: its evolution and domestic breeding in West Africa, transport of the vector to tropical America via the slave trade, and redirection of the vector to Europe and Asia, introducing all four DENV serotypes[2].

The global incidence of dengue has increased exponentially in recent years, with nearly half of the world's population at risk of contracting it. It is estimated that between 100 and 400 million new infections occur annually, of which only 96 million are symptomatic cases. However, this figure may vary due to underreporting and inadequate public health surveillance systems in most tropical countries. It is crucial to consider the conclusion of the systematic review by Gwee et al. in 2021, which highlighted the high potential for global dengue outbreaks, particularly in non-endemic regions with susceptible populations. Additionally, vector control programs may have little or no effect on dengue incidence due to the expanding Aedes mosquito population worldwide, associated with changing climatic conditions and globalization (Castilloa *et al.,* 2011). Moreover, successful programs face sustainability challenges, making dengue an emerging threat to global health security[3,4]. Dengue poses a significant economic burden due to healthcare costs, loss of work hours, and vector control efforts (Humayoun *et al.,* 2010). Given that dengue treatment is supportive, focusing on symptom management, and considering the absence of specific treatments for the infection (Duyen *et al.,* 2011), the development of dengue vaccines has become a priority. Currently, one vaccine is licensed by the WHO, and another is in the process of being licensed for human use. The first vaccine, Dengvaxia® (CYD-TDV), is a live attenuated tetravalent chimeric vaccine, authorized for human use in several countries. However, low efficacy has been observed in children and individuals without prior dengue exposure, with an increased risk of severe dengue in those without previous exposure[5-7]. Therefore, it is essential to pursue new drugs targeting specific viral or cellular components while also emphasizing the importance of accurate and timely diagnosis[8-10].

Dengue can present with various clinical manifestations, ranging from asymptomatic cases to clinically apparent illnesses, which can range from mild, self-limiting febrile episodes to severe dengue. However, a small proportion of infections progress to severe dengue, which can be life-threatening and is characterized by increased capillary permeability, leading to plasma leakage and shock. Severe dengue results in endothelial dysfunction and increased vascular permeability, typically accompanied by altered vascular hemostasis and thrombocytopenia[5-7]. This review aims to present the main clinical and laboratory findings that demonstrate the importance of endothelial activation in the pathogenesis of dengue fever and, based on these findings, to focus on the therapeutic possibilities that are opening up for the management of dengue fever.

Dengue virus (DENV)

The dengue virus belongs to the *Flaviviridae* family and *Flavivirus* genus, which includes over 70 viruses, many of which are transmitted by arthropods. Flaviviruses contain a lipid envelope and nucleocapsid with a single-stranded positive-sense RNA genome[11]. There are four serotypes of the dengue virus, which are antigenically related but genetically distinct (DENV 1-4)[12].

Structural Characteristics

The dengue virion is spherical and approximately 50 nm in diameter. Its genome is a single-stranded positive-sense RNA with an approximate length of 11 kb, composed of 5′ and 3′ untranslated regions (UTR) and a single open reading frame (ORF). This ORF encodes a single polyprotein that is processed by viral and cellular proteases after translation, cleaving it into three structural proteins (C, M, and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5)[13] (Figure 1).

Vascular Endothelium

The endothelium is a monolayer that separates tissues from the blood and lines the lumen of all blood vessels, including arteries, arterioles, capillaries, venules, and veins. Endothelial tissue is composed of endothelial cells. Histologically, this tissue is formed by a single layer of mesenchymal cells with a generally flat appearance; the thickness of these cells can range from 0.1 µm in capillaries and veins to 1 µm in the aorta. Endothelial cells (ECs) have unique cytological characteristics, such as the presence of Weibel-Palade bodies, which are storage granules primarily responsible for storing and releasing von Willebrand factor and P-selectin. Von Willebrand factor is a glycoprotein present in the blood that facilitates platelet adhesion to the surface of a broken vessel, thus participating in the initiation of hemostasis. In contrast, P-selectin is a membrane receptor that plays a role in the rolling of leukocytes, actively participating in hemostasis and inflammation[15]. In adult humans, the vasculature contains approximately 6 × 10^11 endothelial cells, covering a surface area of 4,000 to 7,000 m^2[16].

The endothelium is not merely a barrier between blood and tissues but also an endocrine organ with multiple functions. Its primary roles include regulating systemic flow and per-

fusion through changes in vascular diameter and tone, controlling vascular homeostasis, and managing the passage of substances into cells and tissues. The endothelium actively controls the degree of vascular relaxation and constriction, extravasation of solutes, fluids, macromolecules, hormones, platelets, and blood cells. ECs regulate regional blood flow by regulating vascular tone. They also direct inflammatory cells to regions with foreign materials or areas requiring repair or defense against infections. Additionally, ECs play a critical role in controlling blood fluidity, platelet adhesion and aggregation, and leukocyte activation, adhesion, and transmigration. They maintain a balance between coagulation and fibrinolysis and are crucial for regulating immune responses, inflammation, and angiogenesis[17,18].

ECs are the primary component of the endothelial lining of the circulatory system (blood and lymphatic), heart (chambers and valves), and central nervous system cavities (cerebral ventricles). The tunica intima consists of endothelial cells and a basement membrane, which is the only component of the endothelium in the capillaries. However, in larger vessels (e.g., veins and arteries), the tunica intima is surrounded by a layer known as the tunica media, which consists of smooth muscle cells that regulate characteristics such as vascular distensibility. The tunica media has varying compositions in different parts of the vasculature. Finally, the tunica adventitia is a layer composed of microvasculature within the vessel wall, such as the vasa vasorum[19]. The degree of restriction to access extravascular sites depends on the permeability characteristics of the endothelial cells typical of a specific vascular area under specific pathological conditions, making it a key target for therapeutic interventions in several diseases[19].

Activation and Dysfunction of Vascular Endothelium

Endothelial activation and dysfunction play significant roles in the development of atherosclerosis, cardiovascular diseases (CVD) (e.g., such as coronary artery disease, carotid artery disease, peripheral artery disease, and ischemic stroke), and metabolic syndrome[20]. Endothelial activation promotes atherosclerosis by increasing the permeability of endothelial cell membranes, allowing low-density lipoprotein (LDL) to pass into the intimal layer of blood vessels. Once inside this layer, lipoproteins undergo modification and eventually oxidize, exhibiting pro-inflammatory characteristics that lead to greater adhesion of leukocytes and other molecules to the endothelial surface, ultimately resulting in atherosclerosis (21). Endothelial dysfunction, on the other hand, is a combination of pathophysiological changes in the structure and function of endothelial cells (ECs). Under normal conditions, there is a balance between vasodilatory, antithrombotic, and antiproliferative substances and vasoconstrictive, prothrombotic, and proliferative substances. However, during dysfunction, this balance is disrupted, leading to an endothelial state characterized by phenotypic and functional alterations, where procoagulant, pro-inflammatory, pro-oxidant, and proliferative activities predominate. This state favors all stages of atherogenesis and is marked by reduced production or availability of nitric oxide (NO).

It is important to note that, initially, there is a pro-inflammatory activation, which is characteristic of the nonspecific immune response to viral infections such as dengue. This is followed by endothelial activation, which eventually leads to endothelial dysfunction[18,22].

Mediators of endothelial activation include inflammatory cytokines, such as TNF-alpha, interleukins, oxidized LDL, angiotensin, shear stress, and fibrinogen. The release of Weibel-Palade bodies is also altered, with P-selectin and von Willebrand factor being expressed on the endothelial surfaces. Nuclear factor kappa B (NF-kB), which is responsible for initiating the inflammatory response, plays a fundamental role in this process. NF-kB leads to the expression of inflammatory substances, such as ICAM, VCAM, and various cytokines[23].

Endothelial dysfunction is a central component of several viral syndromes. A common feature of viruses that infect endothelial cells is their ability to cause severe multi-organ

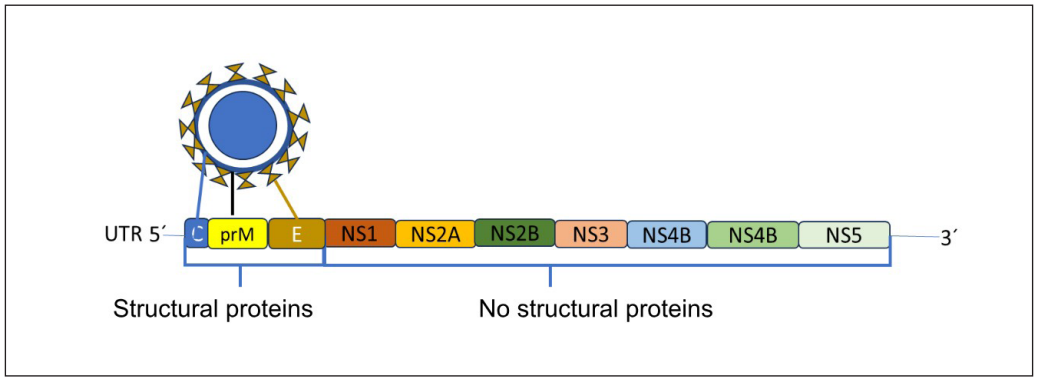

Figure 1. Structure of the Dengue Virus Genome and a Brief Description of Its Proteins. Structural Proteins: C Protein: Involved in genome encapsidation. prM Protein: Functions as a protective cover to prevent premature fusion of the E peptide before viral release. E Protein: Intermediary between the binding and fusion of the virus with the host cell membrane. Non-Structural Proteins: There are 7 non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5. They exhibit various enzymatic activities, but their functional characterization in the infectious cycle is still pending[14].

disease. The clinical characteristics of terminal viral disease are often similar, including hypoperfusion, edema, bleeding, and thrombosis, all of which indicate impairment of central vascular function[24].

Pathophysiology of Dengue and the Role of Endothelial Dysfunction

Current information on the immunopathogenesis of Dengue Hemorrhagic Fever (DHF)/Dengue Shock Syndrome (DSS) remains fragmented. The pathophysiology of DHF is highly complex, involving endothelial activation that allows plasma leakage and triggers the body's homeostatic response, in which the endothelium plays a critical role[25]. Severe forms of dengue often occur as a consequence of secondary infections with a different serotype. Notably, immunity from a single dengue serotype provides protection only against that specific serotype and not against the other three serotypes (Rajapakse *et al.,* 2008). Upon subsequent infection with a different serotype, cross-reactivity with pre-existing antibodies can enhance the viral infection, leading to clinical manifestations of severe dengue, depending on the phenomenon of immune enhancement[26,27].

The morbidity and mortality associated with DHF/DSS are largely due to endothelial barrier dysfunction and vascular leakage syndrome. This hypothesis suggests that direct viral effects on endothelial response to inflammatory mediators and angiogenesis may explain the vascular leakage syndrome of DHF[28]. The target cells include dendritic cells, monocytes, hepatocytes, T lymphocytes, and possibly vascular endothelial cells. The disease results from two main immune mechanisms: the production of non-neutralizing enhancing antibodies that cross-react among DENV serotypes, facilitating viral entry into dendritic cells and monocytes, increasing viral load, and leading to inefficient maturation of infected cells. The other component involves the massive activation of memory T cells sensitized by a prior infection with a different serotype. This activation results in the proliferation and release of pro-inflammatory cytokines and a cytokine cascade targeting susceptible cells, causing apoptosis and contributing to the fluid leakage and liver damage characteristic of DSS/DHF. Basic research on this mechanism is hindered by the lack of an animal model, and few human studies have been conducted. Figure 2 presents a detailed overview of the pathogenesis of DHF/DSS[29].

The current treatment for dengue is largely symptomatic, addressing general discomfort and fever and ensuring adequate hydration. Prevention primarily involves vector control, highlighting the need for a deeper understanding of pathogenesis to develop new molecules that inhibit viral replication and mitigate immune mediator effects (30). For a targeted therapeutic approach, it is crucial to note that endothelial dysfunction, leading to vascular leakage, is a hallmark of severe dengue. This dysfunction typically becomes clinically evident between 3 and 6 days after the onset of illness, known as the critical phase. This phase follows the period of maximal viremia, lasts 24 to 48 h, and generally shows a rapid and complete reversal, suggesting that it is likely mediated by inflammatory factors rather than endothelial infection.

Increased vascular permeability without morphological damage to the capillary endothelium is a key feature of Dengue Hemorrhagic Fever (DHF)/Dengue Shock Syndrome (DSS). Extensive plasma leakage into various tissue spaces and serous cavities, including the pleural, pericardial, and peritoneal cavities, in patients with DHF can lead to profound shock. Various mechanisms have been considered, including immune complex disease, T-cell-mediated mechanisms, cross-reacting antibodies with the vascular endothelium, enhancing antibodies, complement and its products, soluble mediators including cytokines, selection of virulent strains, and viral virulence. However, the most supported mechanism is the enhancement of antibodies and memory T cells during a se-

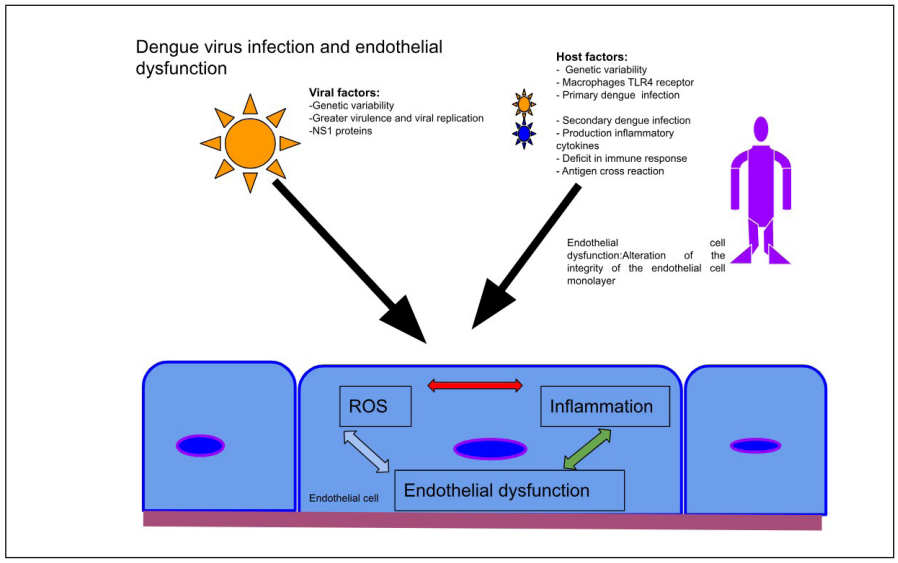

Figure 2. Pathophysiological Mechanisms Involved in Dengue Virus Infection. Adapted from: Bhatt P, 2021[29].

condary infection, leading to a cytokine storm. Regardless of the mechanism, it ultimately targets the vascular endothelium, making it a battleground and leading to severe dengue (Table 1 and Figure 3)[31].

There is no evidence that the virus infects the endothelium, and only minor non-specific changes have been detected in histopathological studies of microvasculature. Although no pathway linking known immunopathogenic substances with definitive events affecting microvascular permeability or thromborregulatory mechanisms has been identified, preliminary data suggest that transient disruption in endothelial function occurs at the level of the endothelial glycocalyx. This layer acts as a molecular sieve, selectively restricting molecules within the plasma based on their size, charge, and shape. During dengue infection, hypoalbuminemia and proteinuria are observed, with proteins up to the size of albumin preferentially lost, consistent with a small but crucial change in the filtration characteristics of the glycocalyx. Both the virus and DENV NS1 protein bind to heparan sulfate, a key structural element of the glycocalyx, and increased urinary excretion of heparan sulfate has been detected in children with severe infection[32].

It is likely that cytokines such as tumor necrosis factor-alpha (TNF-α), which are known to be elevated during the critical phase of dengue, contribute to this condition. The NS1 protein of DENV, a soluble viral protein, has also been shown to alter the endothelial glycocalyx and, therefore, contribute to vascular leakage, although there seems to be a discrepancy between the timing of NS1 antigenemia and the onset of vascular leakage. Moreover, many inflammatory lipid mediators, such as platelet-activating factor (PAF) and leukotrienes, are elevated in acute viral dengue infection. Many other inflammatory mediators, such as vascular endothelial growth factor (VEGF) and angiopoietin-2, have also been found to be elevated in patients with dengue hemorrhagic fever, partially exerting their effects by inducing the activity of phospholipases, which have various inflammatory effects, including PAF generation.

Platelets have also been shown to significantly contribute to endothelial dysfunction through the production of interleukin-1β, which activates the NLRP3 inflammasome, and through the induction of inflammatory cytokine production by monocytes. Drugs that block subsequent immune mediator pathways, such as PAF, may also be beneficial for treating severe disease[33]. Luplertlop et al. (2006) demonstrated that, in a dose-dependent manner, DENV-infected immature dendritic cells induce the overproduction of soluble matrix metalloproteinase (MMP)-9, and to a lesser extent MMP-2, platelet/endothelial cell adhesion molecule-1 (PECAM-1), and the redistribution of actin fibers, which enhances endothelial permeability. These effects were reduced by specific inhibitors and a neutralizing anti-MMP-9 antibody. These in vitro observations were confirmed using an in vivo mouse model of vascular leakage. These results provide a molecular basis for DH/SSD, which could serve as a general model for other hemorrhagic fever-inducing viruses[34].

Table 1. Clinical Effects of Endothelial Cell Dysfunction During Dengue Virus Infection. Adapted from Basu *et al.* 2008[31].

Organ	Effects on Endothelium	Clinical Effects
Pulmonary Pleura	Increased vascular permeability	Pleural effusion
Pulmonary Alveolus		Hemoptysis
Pericardium		Pericardial effusion
Abdomen		Ascites
Brain	Damage to the blood-brain barrier	
Intestine	Increased vascular permeability	Hemoptysis, Melena
Urinary Tract		Hematuria
Female Reproductive Tract		Menorrhagia or hypermenorrhea

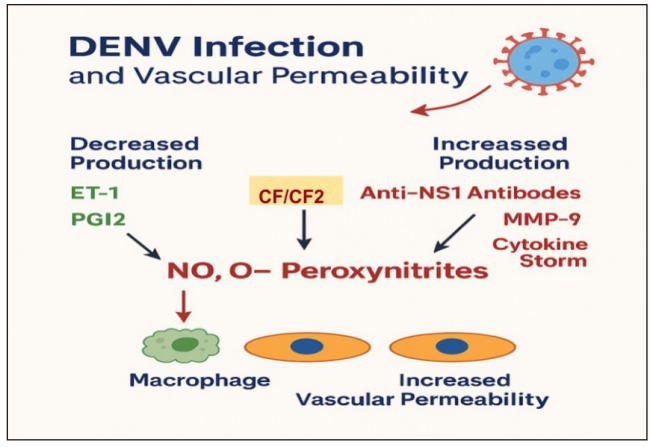

Figure 3. Schematic Representation of the Possible Interaction Between Dengue Virus, Cytokines, and Host Factors, Leading to Endothelial Cell Pathology. (ET-1: Endothelin-1; PGI2: Prostaglandin I2; CF/CF2: Cytotoxic Factor Produced by CD4+ T Cells; Anti-NS1 Antibodies: Antibodies Against NS1 Protein; MMP 9: Matrix Metalloproteinase 9). Adapted from Basu et al. 2008[31].

Basu et al. indicated that the release of cytokines and other inflammatory mediators by memory T lymphocytes acts on the endothelium, resulting in the opening of tight junctions between endothelial cells, which increases vascular permeability[31].

In contrast, Srikiatkhachorn et al. (2015) proposed the following scenario for the progression of plasma leakage and endothelial involvement during DENV infection: (a) acute phase (pre-leakage): the virus infects monocytes, dendritic cells (DCs), macrophages (and possibly endothelial cells), leading to an increase in viremia. Infected cells produce chemokines, such as IL-8 and MCP-1, and cytokines, including TNF-α, which trigger an innate immune response. DENV NS1 is expressed on the surface of infected cells and can be detected in blood. Additionally, angiopoietin (ang)-1, ang-2, VEGF, and VEGFR 2 are found in the blood, and ICAM and VCAM are expressed on the surface of endothelial cells. During this early phase, the endothelium remains intact, and there is no plasma leakage. (b) Plasma leakage phase: Soluble NS1 and NS1/anti-NS1 immune complexes interact with the endothelium and activate the complement system. Cytokines such as TNF-α, MIP-1β, and IFN-γ, as well as other permeability-in-

creasing mediators, are produced by DENV-infected memory cells. sVEGFR2 levels correlated with the degree of plasma leakage, and Ang-1 may antagonize the permeability-enhancing effects of VEGF. DENV-induced secretion of matrix metalloproteinases (MMPs) by DCs may damage endothelial cells, while differential expression of ICAM and VCAM between inactive and active endothelial cells may influence the adhesion and transmigration of circulating leukocytes, which can alter plasma leakage. Circulating levels of sICAM and sVCAM provide evidence of endothelial cell activation and damage. The net result of this scenario is compromised endothelial cells and a weakened barrier, leading to the leakage of albumin-rich fluid into the serous cavities[35].

Regarding the role of NS1 protein in the pathophysiology of severe dengue, the contribution of secreted NS1 from flavivirus to viral pathogenesis remains unclear. However, NS1, a secreted glycoprotein involved in viral replication, immune evasion, and vascular leakage during dengue virus infection, plays a significant role. Puerta-Guardo et al. (2019) demonstrated that NS1 from dengue, Zika, West Nile, Japanese encephalitis, and yellow fever viruses selectively binds to and alters the permeability of human endothelial cells from the lung, dermis, umbilical vein, brain, and liver in vitro. Each flavivirus NS1 induces differential changes in the components of the endothelial glycocalyx, resulting in endothelial hyperpermeability. These findings reveal the ability of a secreted viral protein to modulate endothelial barrier function in a tissue-specific manner both in vitro and in vivo, potentially influencing virus dissemination and pathogenesis and providing targets for antiviral therapies and vaccine development[36].

Additionally, Puerta-Guardo et al. (2016)[37] demonstrated that DENV NS1 alters the endothelial glycocalyx (EGL) in human pulmonary microvascular endothelial cells by inducing sialic acid degradation and the removal of heparan sulfate proteoglycans. This effect is mediated by NS1-induced sialidase and heparanase expression. NS1 also activates cathepsin L, a lysosomal cysteine protease, in endothelial cells, which activates heparanase through enzymatic cleavage. Specific inhibitors of sialidases, heparanase, and cathepsin L prevented the alteration of EGL and endothelial hyperpermeability induced by DENV NS1.

All these effects are specific to NS1 from DENV 1-4 and are not induced by NS1 from the West Nile virus, a related flavivirus. Taken together, these data suggest a significant role for EGL alteration in endothelial dysfunction mediated by DENV NS1 during severe dengue disease[37].

Following this line of research, Glasner et al. (2017) demonstrated in vitro that DENV NS1, but not the closely related West Nile virus NS1, triggers localized vascular leakage in the dorsal dermis of wild-type C57BL/6 mice. Additionally, in vitro studies have shown that human dermal endothelial cells exposed to DENV NS1 do not produce inflammatory cytokines (TNF-α, IL-6, IL-8), and that blocking these cytokines does not affect the endothelial hyperpermeability induced by DENV NS1. Furthermore, it was demonstrated that DENV NS1 induces vascular leakage in mice deficient in TLR4 or TNF-α receptors at levels similar to those in wild-type animals. Finally, this study showed that DENV NS1-induced vascular leakage in vivo can be blocked by using inhibitors targeting molecules involved in glycocalyx alteration. Overall, these data indicate that intrinsic vascular leakage in endothelial cells induced by DENV NS1 is independent of inflammatory cytokines but dependent on endothelial glycocalyx components[38].

Barbachano-Guerrero et al., using primary endothelial cells and a variety of in vitro approaches to study the effect of NS1 on human ECs, demonstrated through confocal microscopy a rapid internalization of NS1 by ECs into endosomes, with accumulation over time. Transcriptomic and pathway analyses revealed significant changes in the functions associated with EC homeostasis and vascular permeability. The functional importance of this activation was assessed using trans endothelial electrical resistance measurements, which showed that NS1 induced a rapid and transient loss of barrier function within 3 h of treatment.

To elucidate the molecular mechanism by which NS1 induces EC activation, we evaluated the p38 MAPK pathway, which is known to be directly involved in EC permeability and inflammation. Western blot analysis of NS1-stimulated ECs showed clear activation of p38 MAPK and its downstream effectors MAPKAPK2 and HSP27, and chemical inhibition of the p38 MAPK pathway restored barrier function. These results suggest that DENV NS1 may be involved in the pathogenesis of severe dengue by activating p38 MAPK in ECs, thereby promoting the increased permeability characteristic of severe disease[39].

Pan et al. (2021) revealed a distinct mechanism by which DENV induces increased endothelial permeability and vascular leakage in human endothelial cells and mouse tissues. It was initially shown that DENV-2 promotes the expression and secretion of matrix metalloproteinase-9 (MMP-9) in the serum, peripheral blood mononuclear cells (PBMCs), and macrophages of patients with dengue hemorrhagic fever (DHF). This study further revealed that DENV NS1 induces MMP-9 expression through activation of the nuclear factor kappa B (NF-κB) signaling pathway. Additionally, NS1 enhances MMP-9 enzymatic activity, which disrupts endothelial cell adhesion and tight junctions, leading to vascular leakage in both human and mouse tissues. NS1 also recruits MMP-9 to interact with β-catenin and zonula occludens proteins 1/2 (ZO-1 and ZO-2) to degrade adhesion and tight junction proteins, thereby inducing endothelial hyperpermeability and vascular leakage. These findings suggest that DENV NS1 and MMP-9 cooperatively induce vascular leakage by altering endothelial cell adhesion and tight junctions, indicating that MMP-9 could be a potential target for treating hypovolemia in patients with DSS/DHF[40].

Furthermore, Lien et al. (2021) examined endothelial cell death induced by treatment with NS1 and domain III (EIII) of the DENV envelope protein, in vitro. Notably, pyroptosis, the primary type of endothelial cell death, was observed, and this effect was attenuated by treatment with Nlrp3 inflammaso-

me inhibitors. Injection of EIII effectively induced endothelial anomalies, and sequential injection of autoantibodies against EIII and DENV-NS1 caused further vascular damage, liver dysfunction, thrombocytopenia, and hemorrhage, which are typical manifestations of dengue hemorrhagic fever or severe dengue. Under the same treatments, physiological changes in Nlrp3 inflammasome-deficient mice were significantly reduced compared to those in wild-type mice. These results suggest that the Nlrp3 inflammasome is a potential therapeutic target for treating DENV-induced hemorrhage in dengue hemorrhagic fever[41].

Cipitelli et al. (2022) reported that acute DENV infection leads to an increase in the secretion of inflammatory mediators, but only IL-10 allowed for distinguishing between mild to moderate dengue cases, suggesting its potential role in aiding clinical prognosis. They also observed that decreased expression of CD147 (basigin) in endothelial cells (ECs) and low levels of MMP-9 in the serum of patients, along with various inflammatory mediator profiles, were associated with the maintenance or loss of monolayer integrity in ECs. These findings are still preliminary, but they point to the need to understand whether there is a subset of cytokines and chemokines that, depending on their combination, could induce opposing effects on endothelial permeability and consequently affect the clinical progression of patients[42].

Finally, several authors have postulated the role of high mobility group box 1 (HMGB1) protein in endothelial dysfunction and dengue. Among the functions of this molecule are the regulation of transcription, cellular activation, and induction of a pro-inflammatory response, which may be involved in endothelial dysfunction. High concentrations of HMGB1 have been detected in patients with several infectious diseases, including dengue, and it could be considered a biomarker for the early diagnosis of dengue and a predictor of complications of the disease[43]. HMGB1-mediated response and raised concerns regarding the participation of this cytokine in promoting or perpetuating inflammation in severe dengue. Oliveira et al. reported in situ evidence of the participation of HMGB1 in severe dengue and highlighted novel considerations in the development of dengue immunopathogenesis (44).Zainal et al. mentioned that HMGB1 migrates out of the nucleus during DENV infection, and this migration is inhibited by RESV treatment and mediated by the induction of Sirt1, which leads to the retention of HMGB1 in the nucleus and consequently helps in the increased production of interferon-stimulated genes (ISGs). The enhanced transcription of ISGs by nuclear HMGB1 contributes to the antiviral activity of RESV against DENV[45]. Chaudhary et al, observed that DENV-2 induces cytoplasmic translocation and secretion of HMGB1. Interestingly, inhibition of HMGB1 secretion by ethyl pyruvate (EP) enhanced viral propagation, whereas silencing of HMGB1 resulted in the abrogation of viral replication in DENV-2 infected A549 cells[46]. suggested that HMGB1 induces BECN1 dependent autophagy to promote DENV-2 replication[47]. And Kamau et al, attempted to elucidate whether the HMGB1-mediated inflammatory response contributes to the pathogenesis of dengue virus (DENV) infection, your data showed that HMGB1 regulated tumor necrosis factor alpha, interleukin. (IL)-6, IL-8, and alpha interferon secretion in DENV-infected DCs. Additionally, increased HMGB1 production was associated with reduced DENV replication titers in DCs. These results suggest that HMGB1 production influences DENV infection in susceptible hosts[48].

Therapeutic Possibilities for Vascular Endothelial Protection in Dengue

It is well established that inflammatory activation and endothelial dysfunction are often key chronic processes in the development and pathophysiology of atherosclerosis and are associated with an increased risk of cardiovascular events. However, in acute viral infections, endothelial cell injury can lead to diffuse and systemic endothelial dysfunction and activation of multiple immune-mediated, thrombotic, and inflammatory pathways, potentially causing severe multiorgan involvement and subsequent morbidity and mortality, see figure 4[49,50].

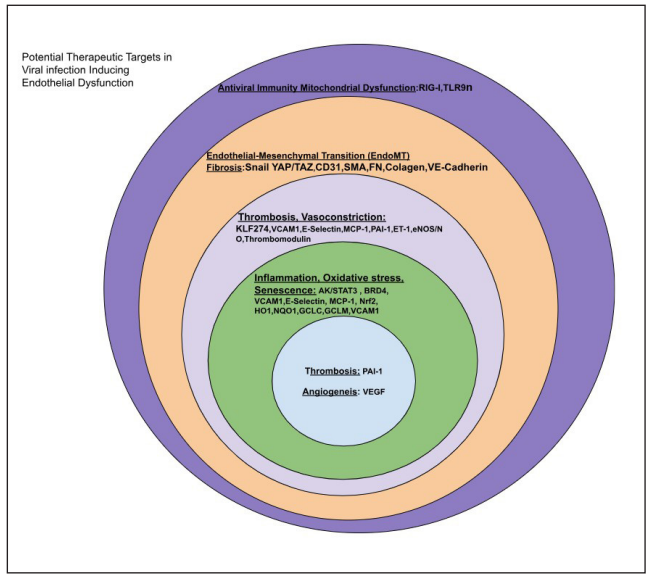

Figure 4. Schematic Representation of Potential Therapeutic Targets in COVID-19 Inducing Endothelial Dysfunction. These targets aim to improve oxidative stress, endothelial inflammation/inflammasome, senescence, fibrosis, cell death, thrombosis, coagulopathy, angiogenesis, endoMT, and immune mechanisms. BRD4: Bromodomain Containing Protein 4; CD31: Cluster of Differentiation 31; CXCL: Chemokine Ligands (C-X-C Motif); EndoMT: Endothelial-to-Mesenchymal Transition; eNOS: Endothelial Nitric Oxide Synthase; ET-1: Endothelin-1; FN: Fibronectin; GCLC: Glutamate-Cysteine Ligase Catalytic Subunit; GCLM: Glutamate-Cysteine Ligase Modifier Subunit; HO-1: Heme Oxygenase-1; IL-1β: Interleukin-1β; IL-6: Interleukin-6; JAK: Janus Kinase; KLF2: Krüppel-Like Factor 2; MCP-1: Monocyte Chemoattractant Protein-1; NF-κB: Nuclear Factor Kappa B; NLRP3: NOD-Like Receptor Family Pyrin Domain Containing 3; NO: Nitric Oxide; NQO1: NAD(P)H Quinone Oxidoreductase 1; Nrf2: Nuclear Factor Erythroid 2-Related Factor 2; PAI-1: Plasminogen Activator Inhibitor-1; RIG-I: Retinoic Acid-Inducible Gene I; RIPK3: Receptor-Interacting Protein Kinase 3; SMA: Smooth Muscle Actin; STAT3: Signal Transducer and Activator of Transcription 3; TLR: Toll-Like Receptor; TLR9: Toll-Like Receptor 9; TNF-α: Tumor Necrosis Factor Alpha; VCAM1: Vascular Cell Adhesion Molecule 1; VEGF: Vascular Endothelial Growth Factor. Adapted from Xu et al. 2023[49].

Endothelial dysfunction is a central component of many viral syndromes, as a common feature of viruses that infect endothelial cells is their ability to cause severe multi-organ disease. The clinical features of terminal viral disease often present similarly, with hypoperfusion, edema, bleeding, and thrombosis, all indicative of impaired central vascular functions[51]. Thus, a common strategy for seeking therapeutic possibilities for vascular endothelial protection, applicable to both cardiovascular diseases and viral infections, may involve interventions targeting endothelial function, such as therapies directed at endothelial cells. Positive-sense single-stranded RNA viruses include clinically significant pathogens that can be lethal in host-specific conditions. For example, SARS-CoV-2 and DENV exploit host factors to access, invade, and replicate within the cells. Studies suggest that these RNA viruses utilize oxidative processes during cell entry, with the release of free radicals due to oxidative stress considered crucial for viral pathogenesis, vascular dysfunction, and endothelial integrity. Given that the vascular endothelium is the primary target of inflammatory processes, antioxidant therapy could be further explored as a supportive treatment for RNA virus-induced diseases[52].

Moreover, these RNA viruses can provoke endothelial activation, endothelial dysfunction, increased vascular permeability, inflammation, and subsequent activation of the innate immune response, which can lead to a cytokine storm (see Figure 5)[50-53]. Lipskaia et al. concluded a phase 2 trial demonstrating the safety and benefit of transferring the Ca2+ ATPase pump gene (SERCA2a) via adeno-associated virus type 1 in advanced heart failure, suggesting that when administered via perfusion, the viral vector carrying SERCA2a may also transduce vascular endothelial cells and smooth muscle cells (ECs and SMCs), thereby enhancing the clinical benefit of gene therapy. This indicates that it could be an option for treating vascular dysfunction[54].

During the COVID-19 pandemic, numerous therapeutic studies targeting endothelial dysfunction were conducted, including hypolipidemic drugs, antihypertensive agents such as angiotensin-converting enzyme-2 (ACE2) inhibitors, angiotensin receptor blockers (ARBs), antidiabetic drugs, anti-VEGF agents, anticoagulants, antioxidants, anti-inflammatory drugs, bromodomain-containing protein 4 inhibitors (BRD4i), Janus kinase (JAK) inhibitors, sodium-glucose cotransporter 2 inhibitors (SGLT-2i), among others. These treatments could be considered for future clinical studies on other RNA viruses, such as DENV[55]. See Table 2.

Studies have demonstrated that curcumin (Curcuma longa) has significant interactions with endothelial cells and acts as an effective therapeutic agent for the regulation of endothelial function[53]. In addition, silencing of HMGB1 showed a reduction in BECN1 and stabilization of BCL-2 protein, showing that the modulation of autophagy by DENV-2 is HMGB1/BECN1 dependent[47] Finally, it has been proven that RESV antagonizes DENV replication and that nuclear HMGB1 plays a role in regulating ISG production[45].

In conclusion, the precise mechanisms leading to the manifestations that determine severe dengue infection remain unclear, but they are believed to be multifactorial, involving

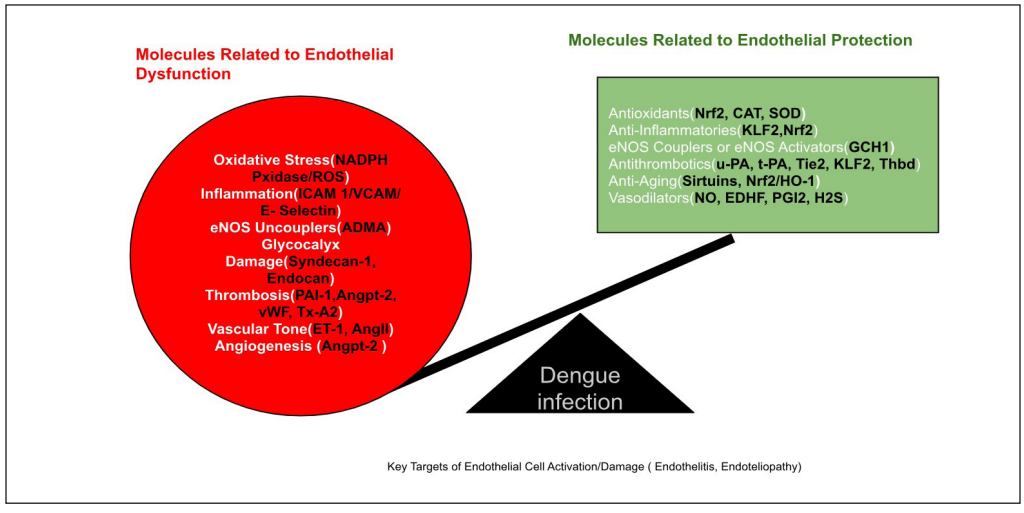

Figure 5. Schematic Representation of Key Targets of Endothelial Cell Activation by SARS-CoV-2 Compared to Dengue Virus Infection in this article. Viral infection disrupts the balance between endothelial protective and damaging molecules, leading to endothelial dysfunction. ADMA: Asymmetric Dimethylarginine, Ang II: Angiotensin II, Angpt-2: Angiopoietin-2, CAT: Catalase, EDHF: Endothelium-Derived Hyperpolarizing Factor, eNOS: Endothelial Nitric Oxide Synthase, ET-1: Endothelin-1, GCH1: GTP Cyclohydrolase 1, H2S: Hydrogen Sulfide, HO-1: Heme Oxygenase-1, ICAM-1: Intercellular Adhesion Molecule 1, KLF2: Krüppel-Like Factor 2, NO: Nitric Oxide, Nrf2: Nuclear Factor Erythroid 2-Related Factor 2, PAI-1: Plasminogen Activator Inhibitor-1, PGI2: Prostaglandin I2, ROS: Reactive Oxygen Species, SOD: Superoxide Dismutase, TF: Tissue Factor, Thbd: Thrombomodulin, Tie-2: Tyrosine Kinase Receptor, tPA: Tissue Plasminogen Activator, Tx-A2: Thromboxane A2, uPA: Urokinase-Type Plasminogen Activator, VCAM-1: Vascular Cell Adhesion Molecule 1, vWF: von Willebrand Factor. Adapted from Xu et al. 2023[55].

Table 2. Endothelial Protective Medications with Protective Effects Against Endothelial Dysfunction in COVID-19.

Lipid-Lowering and Antihypertensive Agents	Hypoglycemics	Anticoagulants	Anti-Inflammatories	Others
• Statins • ACE Inhibitors • Angiotensin 2 Receptor Blockers • Spironolacton • Galectin 3 Inhibitors	• SGLT2i • Metformin **Anti-VEGF** • Bevacizumab **Glycocalyx Stabilizers** • Sulodexide • Heparan Sulfate	• Heparin • Aspirin **Antioxidants** • Vitamin C • Traditional Chinese Medicine	• Canakinumab • Anakinra • Tocilizuma • Colchicine • Dexamethasone • JAK Inhibitors • CCR5 Blockers • BRD4 Inhibitors (AZDG153) • Adrecizumab	• 17β-Estradiol • AKB-9778 • Tie2 Activator • L-Arginine • NO Donors • Fluvoxamine • PGI2 Agonist (Iloprost) • Senolytics • Mesenchymal Stem Cells

Adapted from: Xu SW, 2023[49].

both viral characteristics and host factors. These factors include prior immunity to the infecting serotype, viral load, and the presence of anti-NS1 antibodies, among others, which contribute to increased capillary permeability and disease severity[56].

Excessive inflammatory responses are associated with changes in endothelial cells in blood vessels, resulting in a spectrum of disease severities. These effects are driven by the host's immune response to DENV infection, leading to fluid loss, bleeding from mucosal surfaces, and gastrointestinal tract bleeding. In such cases, vascular permeability induced by DENV can result in hypovolemic shock, hemoconcentration, and disseminated intravascular coagulation. Currently, no specific antiviral treatments are available, and only supportive measures are used to manage the syndrome resulting from endothelial injury[57]. Therefore, a therapeutic approach to DENV infection is needed, focusing on modulating the inflammatory process and its mediators, which ultimately induce endothelial activation and dysfunction, responsible for severe manifestations and mortality associated with the virus.

Ethical considerations

Since this is a bibliographic review article in databases, ethical considerations such as those for Protection of persons, Protection of vulnerable populations, Confidentiality and Privacy do not apply.

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Conflict of interests. The authors have no conflict of interest to declare.

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Authors´ contribution. SG: JM, MG, JC: collected the paper and wrote the paper. All authors contributed to read and approved the version of the submitted manuscript.

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