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# Bidirectional Regulation of Nitric Oxide and Endothelin-1 in Cerebral Vasospasm: Mechanisms and Therapeutic Perspectives

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## Abstract

Cerebral vasospasm (CVS) following a subarachnoid hemorrhage (SAH) is a critical complication driven by imbalances between vasodilators and vasoconstrictors. This review explores the bidirectional interplay between nitric oxide (NO) and endothelin-1 (ET-1) in CVS pathogenesis. NO, a potent vasodilator mainly produced by endothelial and neuronal nitric oxide synthase (eNOS/nNOS) under normal physiological conditions, is scavenged early after SAH by hemoglobin derivatives, leading to microcirculatory dysfunction, pericyte constriction, and impaired neurovascular coupling. Conversely, ET-1 exacerbates vasoconstriction by suppressing NO synthesis via ROS-dependent eNOS uncoupling and Rho-kinase activation. The NO/ET-1 axis further influences delayed cerebral ischemia (DCI) through mechanisms like 20-HETE-mediated cGMP suppression and oxidative stress. Emerging therapies—including NO donors, NOS gene therapy, and ET-1 receptor antagonists—aim to restore this balance. Understanding these pathways offers translational potential for mitigating CVS and improving outcomes post-SAH.

**Keywords:** cerebral vasospasm; nitric oxide; endothelin-1; subarachnoid hemorrhage

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## 1. Introduction

Cerebral vasospasm (CVS), which is the widespread constriction of the cerebral vasculature, represents a devastating complication following a subarachnoid hemorrhage (SAH) that significantly contributes to delayed cerebral ischemia (DCI) and poor neurological outcomes. Clinical CVS, diagnosed based on the manifestation of delayed ischemic neurological deficits or reduced territorial levels of CBF in combination with CVS in angiography [1], is also present in up to 16% of angiographically negative cases [2]. However, those that present with more severe pathophysiology and neurological deficits [3,4] and angiographically visible vasospasm do not necessarily include clinical vasospasm [2]. CVS is biphasic, with a delayed phase after 3–5 days and a maximum on day 7, accompanied by delayed cerebral ischemia [5–9]. Despite decades of research, its pathogenesis remains incompletely elucidated. Several mechanisms contribute to the development of CVS. Blood-derived factors play a central role, including the presence of blood clots [10–12], hemoglobin [13–15], lysed erythrocytes [16,17], and the release of free iron [18]. These compounds trigger a cascade of oxidative and metabolic processes, such as the oxidation of bilirubin [19], activation of heme oxygenase-1 (HO-1) [19], and oxidative stress [20], with superoxide-induced lipid peroxidation further amplifying vascular injury [14,21]. In parallel, inflammatory pathways

are strongly implicated [22–26]. Structural and cellular changes also contribute, including the stretching of the arachnoid membrane [27], abnormal contraction of smooth muscle cells, perivascular neuron cell death, remodeling of the arterial wall [9,28,29], and apoptosis [30–32]. A variety of messenger molecules and signaling mediators further exacerbate CVS. These include serotonin (5-HT) and endothelin-1 (ET-1) [33,34], prostaglandins [34,35], and sympathetic activation through catecholamines and neuropeptide Y [9,27,34–36], as well as other components of the vascular innervation [9,14,37]. Finally, several molecular and lipid signaling pathways have been implicated, such as calcium channel activation in smooth muscle cells [37], sphingolipids (probably derived from thrombocytes) [22], thromboxane [38], MAPK and ERK1/2 signaling [39], and the statin-mediated P3K/Akt/eNOS pathway [40].

Two predominant mechanistic hypotheses dominate the current understanding: nitric oxide (NO) dysregulation and ET-1 overactivation or intracellular signal transduction [41]. Briefly, for the NO hypothesis, early NO depletion due to scavenging by the hemoglobin present in the subarachnoid space after SAH impairs endogenous vasodilation, triggering microcirculatory failure. This loss disrupts the sGC-cGMP (soluble guanylate cyclase and cyclic guanosine monophosphate) pathway and 20-hydroxyeicosatetraenoic acid (20-HETE)-mediated calcium signaling, leading to sustained pericyte constriction and capillary flow arrest. For the ET-1 overactivation hypothesis, ET-1, a potent vasoconstrictor, is upregulated post-SAH through transcriptional activation and mitogen-activated protein kinase (MAPK) phosphorylation, both depending on reactive oxygen species (ROS) as another mediator derived from the hemorrhage in the subarachnoid space. It can directly antagonize NO by suppressing eNOS activity via Rho-kinase (ROCK), while concurrently promoting vascular smooth muscle cell (VSMC) hypercontractility. Notably, although endogenous vasodilation is widely recognized as essential to vascular homeostasis and angiogenesis [41], currently the putative importance of the NO/ET-1 axis and the modulatory roles of intracellular signaling pathways (particularly MAPK in ET-1 receptor trafficking and phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) in NOS phosphorylation) are still not fully emphasized [41].

This review synthesizes advances in the bidirectional NO/ET-1 interplay in cerebral vasospasm pathogenesis, focusing on three critical dimensions: (1) the molecular drivers of axis disruption, including ROS-mediated eNOS uncoupling, 20-HETE-dependent suppression of NO-cGMP signaling, and underemphasized intracellular pathways (MAPK/PI3K-Akt) regulating ET-1 trafficking and NOS phosphorylation; (2) microcirculatory sequelae, spanning pericyte α-SMA (alpha smooth muscle actin) transformation, capillary flow arrest, and cortical spreading depolarizations (CSDs) that amplify delayed cerebral ischemia (DCI); and (3) emerging pharmacotherapies targeting axis recalibration, from hypoxia-activated NO donors and ET-1 antagonists (e.g., clazosentan) to dual-path agents like 20-HETE inhibitors (HET0016).

## 2. NO-Mediated Vasoregulation: Pharmacological Foundations

NO, a critical endothelium-derived relaxing factor, maintains vascular homeostasis by counteracting vasoconstrictors (e.g., angiotensin II, endothelin-1, 5-HT). Its inhibition triggers vasoconstriction and pressor responses [42–45], as evidenced by the 50–75% reductions in angiotensine II-induced vasoconstriction upon 20-HETE synthesis inhibition, and the deletion of eNOS in mice in different cell types, which goes hand in hand with hypertension. NO mediates carbon dioxide ($CO_2$)-dependent vasodilation via the canonical sGC/cGMP pathway [12].

Following the classical view, the vasodilatory response to NO is secondary to the activation of sGC and a subsequent elevation of cGMP [12,44]. NO binding to sGC elevates

cGMP, activating calcium-activated $K^+$ (KCa) channels to sequester cytosolic $Ca^{2+}$ into the sarcoplasmic reticulum, thereby relaxing vascular smooth muscle cells [13,46] and acting as a pericyte dilator [14]. This mechanism underpins $CO_2$-induced vasodilation in cerebral vessels.

However, more recent investigations have shown the importance of the cGMP-independent modulation of NO levels, e.g., through 20-HETE [13,42,44]. This cytochrome P450 (CYP4A)-derived metabolite suppresses NO bioactivity by blocking KCa channels, causing VSMC depolarization and impaired normal SMC relaxation [14,44]. It also modulates GC via P/Q type channels and N-methyl-D-aspartate (NMDA) receptor modulation [47]. Intriguingly, NO reciprocally inhibits 20-HETE synthesis by binding to the heme moiety of CYP4A enzymes—a critical regulatory node where diverse vasoconstrictive pathways (e.g., PKC/Rho-kinase) converge to amplify 20-HETE production [13,44].

The spatiotemporal dynamics of NO signaling are governed by three nitric oxide synthase (NOS) isoforms with distinct pharmacological profiles [18,48]. Under physiological conditions, endothelial NOS (eNOS/NOS3), activated by shear stress or PI3K-Akt phosphorylation, generates NO for sustained vasodilation and pericyte relaxation, serving as the primary regulator of basal vascular tone and vasodilation [15,18,48–51]. However, some studies reported that, in SAH, oxidative stress can lead to "eNOS uncoupling," where the enzyme produces a superoxide instead of NO, thereby exacerbating vascular dysfunction and oxidative injury [52]. Neuronal NOS, formerly named non-inducible NOS (nNOS/NOS1), localized in perivascular neurons and pericytes [14,50], is a constitutive enzyme that mediates neurovascular coupling and central blood pressure regulation. While numerous reports indicate nNOS-derived NO can upregulate cytoprotective antioxidant defenses, some reported that its hyperactivity under a few pathological conditions may contribute to excitotoxic damage [53]. Notably, both eNOS and nNOS are constitutive enzymes, but their activity can be enhanced by various stimuli. Conversely, inducible NOS (iNOS/NOS2) is expressed at low levels under physiological conditions, but is robustly upregulated during inflammation in a wide range of cells post-SAH, including macrophages, microglia, vascular smooth muscle cells, and pericytes [52,54]. The role of iNOS is profoundly dual and context-dependent. On one hand, its high-output NO production can be pathogenic, generating peroxynitrite and driving cerebral vasospasm (CVS) and neuronal apoptosis [52]. On the other hand, iNOS also possesses critical regulatory functions, such as suppressing pathogenic T-cells and promoting the infiltration of regulatory myeloid cells, which can help control neuroinflammation [55].

Under physiological conditions, endothelial-derived NO diffuses to adjacent SMCs (<100 μm), directly mediating VSMC relaxation [18], while neuronal NO (from nNOS) fine-tunes microvascular tone through perivascular nerve plexuses that anatomically bridge nitrogenergic neurons and cerebral microvessels. Notably, during neuronal activity-regulated vasodilation, the close proximity between capillaries and neurons (<50 μm) enables efficient NO signaling despite its limited diffusion range (<200 μm) [47]. However, in pathological contexts such as ischemia–reperfusion (I/R), microvessels become the dominant NO source due to endothelial activation and inflammatory cell infiltration [15]. This shift is functionally critical, as I/R-induced oxidative stress rapidly scavenges NO, exacerbating microcirculatory dysfunction despite increased production.

Pharmacologically, this multifaceted regulation offers actionable targets; in particular, the interplay between NOS isoforms, 20-HETE, and downstream effectors thus defines a druggable axis for vascular pathologies.

## 3. NO Dysregulation Post-SAH: Therapeutic Challenges

NO is critically involved in regulating cerebral blood flow (CBF) after SAH, with its depletion driving disturbances in the microcirculation. These include capillary flow impairment, microvascular constriction at pericyte sites, and reduced mean transit time (MTT), which is closely correlated with decreased NO levels and increased capillary transit time heterogeneity (CTH), alongside large vessel cerebral vasospasm (CVS) [12,14,15,27,44,56–59]. Concurrently, $CO_2$ reactivity diminishes [12], yet Cseplo et al. observed a preserved $CO_2$ reactivity in large vessels despite hemoglobin-induced NO scavenging [46].

NO levels decrease during the early phase post-SAH in both human and animal models [60,61], consistent with its known reduction under inflammatory conditions in general [62]. This depletion post-SAH occurs primarily because extravasated blood scavenges NO via oxygenated hemoglobin (oxyHb), bilirubin, and iron [15,25,44,63,64], resulting in a transient fall in CBF. Endogenous NOS inhibitors and the degeneration of perivascular nitrergic neurons further reduce early NO availability [14,15,44,65,66]. For example, asymmetric dimethyl arginine (ADMA), of which the degradation is inhibited by hemoglobin metabolism (bilirubin-oxidized fragments), inhibits the physiological upregulation of eNOS upon vasoconstriction-mediated shear stress. Notably, while the upregulation of NO products in the brain appears to oppose the sustained vasoconstrictor response to hemoglobin, these factors (ADMA accumulation and neuronal degeneration) act as causatives for the prolonged fall in CBF (30 min until 12 h). Concurrently, tissue hypoxia exacerbates this loss by limiting the oxygen substrate required for NO synthesis [14]. This initiates a vicious cycle: NO depletion leads to hypoxia, which further aggravates NO deficiency. Furthermore, ROS play a central role in the loss of NO bioavailability after SAH. Extravasated blood in the subarachnoid space generates a significant oxidative burden, for instance, through the autoxidation of oxyhemoglobin. Among these ROS, the superoxide anion ($O_2^-$) reacts with NO at an extremely rapid rate, forming the potent oxidant peroxynitrite ($ONOO^-$) [67]. This reaction not only directly scavenges vasoprotective NO but also yields a product that inflicts further damage. Peroxynitrite contributes to a vicious cycle by inducing eNOS uncoupling, likely through the oxidation of the essential eNOS cofactor tetrahydrobiopterin (BH4) [67]. This converts eNOS from a protective NO synthase into a source of additional superoxide, drastically amplifying oxidative stress and depleting NO. Concurrently, the endogenous antioxidant defense systems, including enzymes like superoxide dismutase and the glutathione system, become overwhelmed and deficient [68]. This failure of antioxidant capacity allows ROS to accumulate unchecked, accelerating NO consumption and directly compromising endothelial function. However, in contradiction to observations implicating NOS reduction in prolonged CBF decline, some studies reported no effect of NOS inhibition on CBF [15,44]. In the longer run (12–24 h post-SAH), NO metabolites (nitrite/nitrate) and eNOS increase in the CSF [69], suggesting compensatory mechanisms despite persistent microcirculatory dysfunction.

By day 7 post-SAH, oxygenated hemoglobin (oxyHb) from hemolysis peaks, coinciding with the maximal CVS severity, induces vasoconstriction through the ROS generated during its autoxidation to methemoglobin (metHb) [14,44,69]. Heme degradation by heme oxygenase-1 (HO-1) releases bilirubin, carbon monoxide (CO), and iron, fueling peroxynitrite formation and elevated nitrotyrosine [44]. Peroxynitrite itself acts as a vasoconstrictor by impairing smooth muscle cell relaxation [14,69], inactivating tissue plasminogen activator (tPA) to increase thrombogenicity, and causing tissue damage via lipid peroxidation, poly(ADP-ribose)-polymerase (PARP) activation, and mitochondrial dysfunction [14,70], thereby worsening the post-SAH energy crisis and amplifying ROS production.

Mechanistically, NO depletion inhibits the NO-cGMP pathway, triggering pericyte $\alpha$-SMA transformation and constriction [14,15]. Oxyhemoglobin also mediates vasocon-

striction by altering SMC potassium channels, upregulating voltage-dependent calcium channels, and modulating transient receptor potential channels [63]. Notably, hemoglobin-induced NO scavenging partly stems from increased 20-HETE production; blocking 20-HETE prevents CBF reduction after blood/Hb injection and normalizes vascular responses to NOS inhibition [44]. Similar effects can also be observed after cerebral ischemia [15].

Other reasons for the microcirculatory failure may be due to NO's role in suppressing thrombocyte and neutrophil activation and vascular adhesion [27]—low NO levels correlate with microthrombosis extents [14]. Reduced NO (alongside elevated $K^+$) also lowers the threshold for CSDs. These propagate via P/Q-type calcium channels and NMDA receptor modulation [47,71], potentially driving the inverse haemodynamic response and spreading ischemia after SAH [65,71]. Repetitive CSDs exacerbate microvascular spasms and NO resistance through intracellular $Ca^{2+}$ accumulation or matrix metalloproteinase-9 (MMP-9) activation, mechanistically explaining delayed ischemic neurological deficits (DINDs) [72,73].

Beyond CBF dysregulation, NO contributes to tissue damage [72,73]. While crucial for vasodilation, its overproduction, particularly in reaction with superoxide to form peroxynitrite ($ONOO^-$), can mediate glutamate excitotoxicity and oxidative stress, leading to neuronal death [74,75]. This pathway is implicated in inducing mitochondrial dysfunction and apoptotic signaling [75]. Concurrently, elevated NO can disrupt the blood–brain barrier (BBB), potentially by mediating VEGF-induced increases in permeability [44,75]. Endothelial dysfunction is another component of the tissue damage after SAH, inducing an imbalance in vessel tone regulation [76]. An overproduction of NO via inflammatory induction contributes to cytotoxic and structural damage [14,77].

Paradoxically, NO reduction in the microcirculation links to delayed cerebral ischemia [47,57,78], and NO depletion leads to CSD promotion and cortical spreading ischemia (CSI), which further contribute to the development of DCI and DIND [47,72,79,80], while NO donors confer neuroprotection by sustaining microvascular oxygen delivery during the critical phase post-SAH or ischemia–reperfusion [14].

NOS isoform polymorphisms and dynamics further complicate the pathology and the relevant therapy [81]. nNOS (constitutively cytoprotective) disappears from neuronal fibers in the arterial adventitia after SAH [14], due to hemoglobin-induced neuronal destruction [15]. ET-1 also inhibits nNOS-derived NO, impairing functional hyperemia and neurovascular regulation [82]. eNOS, a major endothelial source of NO, becomes phosphorylated through ROS-induced peroxynitrite-mediated uncoupling after SAH, resulting in reduced NO levels [83]. The main source of NO after brain I/R is the microvessels/capillaries [14,15], with reduced levels of eNOS after SAH [14,80], for which microcirculation-related damages are one probable cause [15], though also an increased production of NO due to an increase in eNOS in the walls of pial arteries has been observed in the first days after SAH, which is followed by eNOS reduction and accompanied by an increase in NO breakdown products [14]. eNOS is also found in pericytes under inflammatory conditions in SAH [15,84], negatively affecting macrophage infiltration in experimental treatments of cerebral aneurysm rupture [84].The effects of eNOS phosphorylation/activation after SAH as well as NO production are still controversial; they cause neuroprotection and injury [15,40]. Due to its vasodilatory effects, eNOS exerts a protective effect in the early stages of ischemia [14,18]. Its dysfunction contributes to the impairment of vasodilation upon SAH [62], rendering it a contributor to the development of CVS [40,44,69,85], e.g., via promoting an increased adherence and infiltration of leukocytes in cerebral arteries [14,80], via p53 inhibiting SMC proliferation [30] and via promoting the alpha-SMA transformation of pericytes, which causes microvessel constriction [15].

Disturbances in the microcirculation are also promoted by increased levels of NO. It also contributes to preserving CBF autoregulation in rat pial arteries in the acute stage after SAH, probably via the scavenging of superoxide anions by NO (lower limit increased, eNOS and superoxide anion increased in hypotension, L-arginine preserved) [86], and reduces the development of neurological deficits after SAH [15]. In contrast to this, the inducible, proinflammatory NOS isoform, iNOS, represents a pivotal and complex player in SAH pathophysiology. Under physiological conditions, iNOS is barely detectable in cerebral vessels [85]. However, following SAH, it is found at increased levels in macrophages and mononuclear and polymorphonuclear cells in the subarachnoid space. In cerebral vessel walls, it is present in SMCs, endothelial cells, and adventitial cells, and under the inflammatory conditions in SAH can furthermore be found in microglia, neurons, leukocytes, thrombocytes, fibroblasts, and pericytes [15,18,48,62,70,84,87]. This expression is driven by a complex inflammatory cascade involving cytokines and master regulators like NF-κB, which is itself activated by ROS and PARP [18,26,48,58,64,87,88]. As previously mentioned, the role of iNOS in pathophysiology is profoundly dual and context-dependent. On one hand, its high-output, sustained production of NO is a well-established pathogenic mechanism. On the other hand, a more nuanced understanding has emerged, revealing that iNOS also possesses critical immunoregulatory functions. This duality explains why a global inhibition of iNOS has shown mixed outcomes, sometimes worsening pathology [55]. Therefore, the therapeutic paradigm is shifting. While iNOS remains a high-value target due to its clear detrimental effects, the strategy is evolving from one of simple blockade to one of fine-tuning, for instance, by ensuring proper cofactor availability to prevent uncoupling.

## 4. ET-1/NO Antagonism: Pharmacodynamic Interplay

Despite conclusive evidence of the NO/ET-1 axis critically regulating vascular homeostasis [85,89], its therapeutic exploitation remains underdeveloped. The bidirectional imbalance between NO and ET-1 constitutes a convergent pathway in cerebral vasospasm pathogenesis, operating through reciprocal molecular suppression (Figure 1). When ET-1 binds $ET_A$ receptors, it activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to generate superoxide ($O_2^-$), which scavenges NO to form peroxynitrite ($ONOO^-$)—a process that oxidizes eNOS cofactors, converting this critical enzyme from an NO synthase to an ROS generator (uncoupling), while increasing nitrotyrosine levels [18,69]. This ET-1-mediated eNOS inactivation occurs through ROCK-dependent phosphorylation changes, simultaneously suppressing neuronal NO production by nNOS and impairing activity-dependent vasodilation (neurovascular uncoupling) [15,82]. This dual inhibition is particularly consequential after SAH, where nNOS disappears from neuronal fibers in the arterial adventitia and nNOS-containing neurons are destroyed by hemoglobin [14]. Compounding this damage, ET-1 inhibits NO production by the surviving nNOS, reducing functional hyperemia and impairing neurovascular regulation [82,90].

The resulting oxidative stress manifests through multiple pathways: ET-1 induces mitochondrial ROS, eNOS uncoupling, and the activation of NADPH oxidase in vascular cells [82]. These effects render the brain more susceptible to injury by compromising the balance between energy demands and blood flow delivery. Crucially, hemoglobin breakdown products (methaemoglobin [metHb], bilirubin) disrupt the ET-1/NO equilibrium by simultaneously scavenging NO and amplifying ET-1 release, establishing a self-perpetuating cycle of vasoconstriction. This imbalance extends beyond macrovascular spasms to drive microcirculatory failure, which is manifested through a pericyte α-SMA transformation and capillary stall via 20-HETE accumulation and ET-1-induced RhoA/ROCK activation.

Conversely, physiological NO suppresses ET-1 transcription through counter-regulatory suppression. It activates the cGMP-PKG (protein kinase G)-mediated inhibition of nuclear factor kappa B (NF-κB) and activating protein-1 (AP-1) signaling cascades, reducing ET-1 gene expression while downregulating vasoconstrictive $ET_A$ receptors and preserving protective $ET_B$ receptors that mediate NO/prostacyclin release. Nerve fiber stimulation exploits this pathway, with NO release relieving angiographic vasospasm. The PI3K/Akt pathway mediates ischemic tolerance by maintaining endothelial cell survival and NO-mediated vascular tone regulation, resulting in reduced vasospasm. This protective mechanism involves eNOS phosphorylation through PI3K/Akt activation (e.g., by statins) [15,40,85] and the DCC (deleted in colorectal cancer)-ERK1/2 (extracellular signal-regulated kinase)-eNOS-NO feed-forward loop after cardiac I/R [15], which counteracts ET-1's damaging effects.

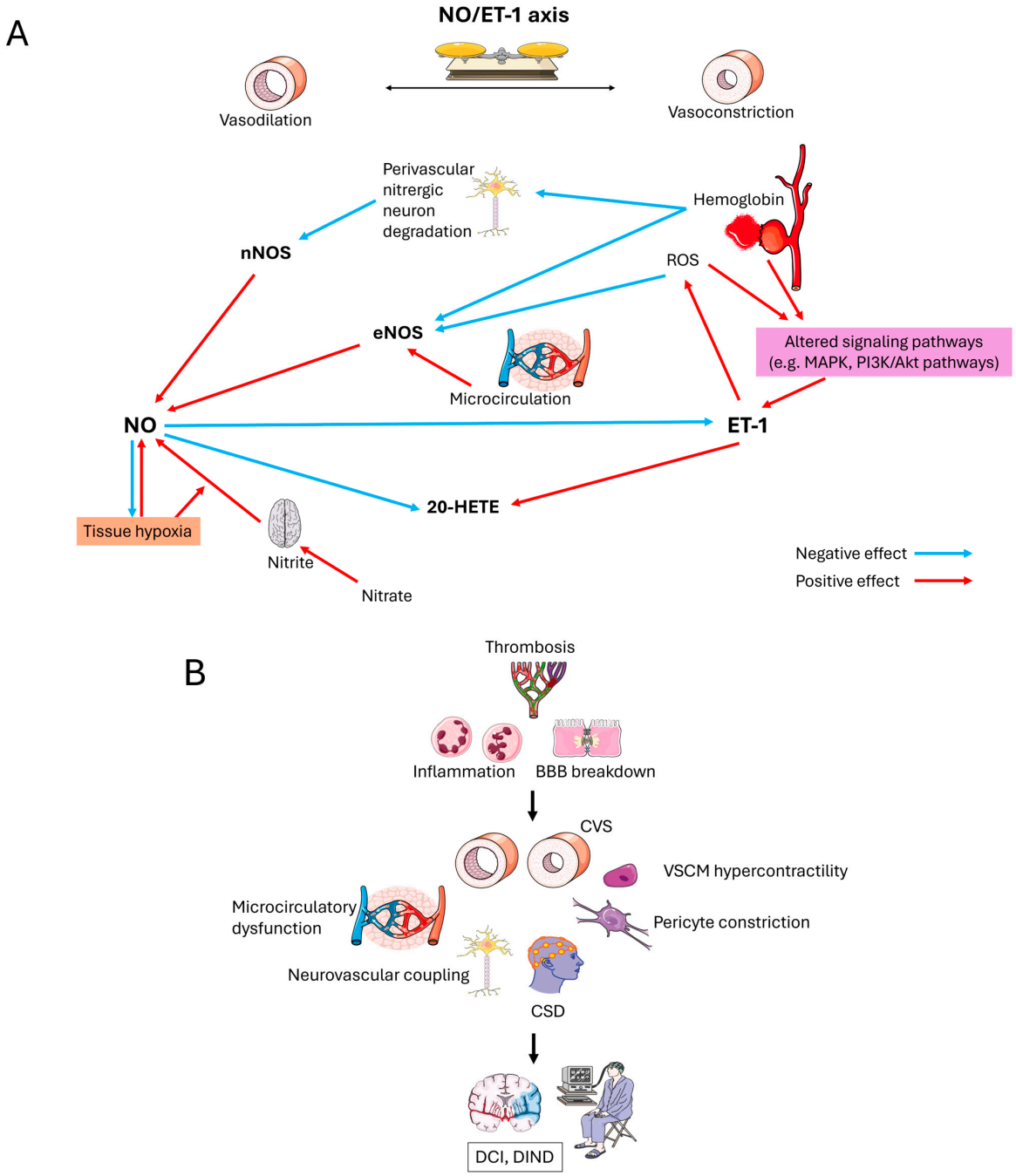


**Figure 1.** *Cont.*

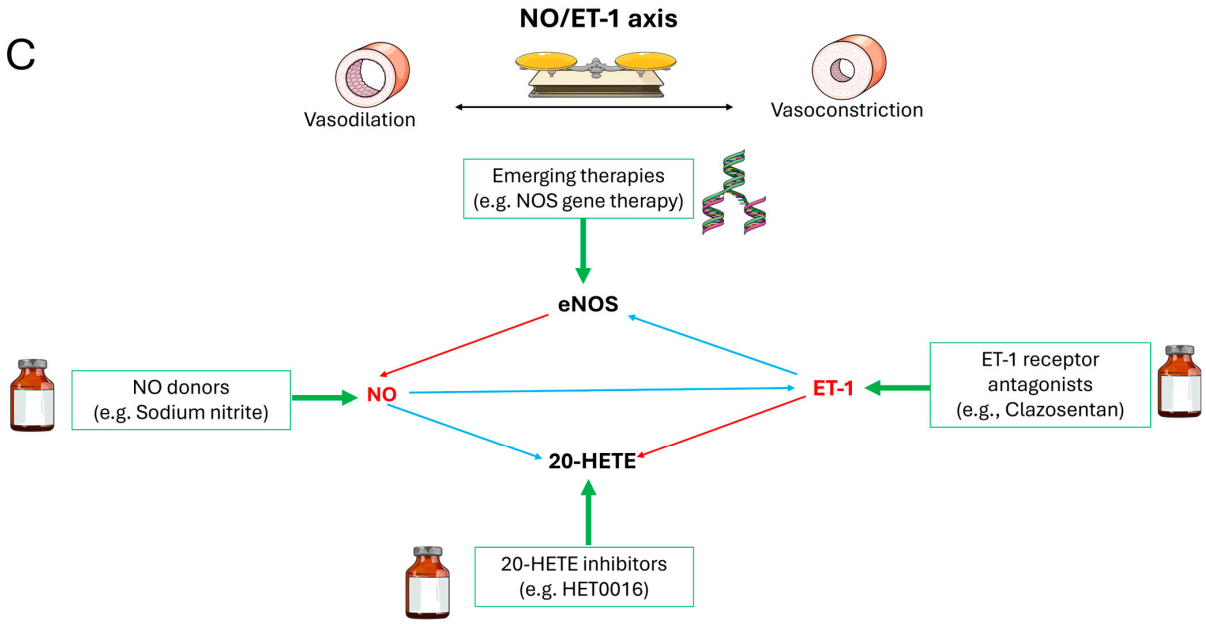


**Figure 1.** Depiction of the relevant NO/ET-1 axis pathophysiological pathways for the development of DCI and DIND following SAH. (**A**) Molecular pathogenesis initiated by SAH. The pathophysiological cascade is characterized by synergistic interactions: the initial insult involves massive scavenging of NO and a surge in reactive oxygen species (ROS), which directly damage endothelium and induce eNOS uncoupling. These processes induce the upregulation of 20-HETE, a key mediator that suppresses residual cGMP-dependent vasodilation. In parallel, ROS and other inflammatory mediators co-activate MAPK and PI3K/Akt pathways, which further exacerbate the imbalance by enhancing vasoconstrictor gene expression (e.g., ET-1) and altering NOS activity, thereby cementing the vicious cycle. (**B**) Pathophysiological cascade in the microvasculature. (**C**) Representative pharmacotherapies for axis recalibration, including hypoxia-activated nitric oxide donors, ET-1 receptor antagonists (e.g., clazosentan), and multi-pathway agents such as 20-HETE synthesis inhibitors (e.g., HET0016) that target convergent mechanisms of vasospasm. Figure was prepared using Servier Medical Art (https://smart.servier.com/ (accessed on 4 September 2025)), licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/ (accessed on 4 September 2025)).

Pathologically, SAH triggers a vicious cycle initiated by hemoglobin breakdown products, oxyHb and bilirubin, that simultaneously scavenge NO and amplify ET-1 secretion. This dual assault drives microcirculatory collapse through ET-1-induced pericyte $\alpha$-SMA transformation (via RhoA/ROCK) and concurrent NO depletion blocking KCa channels, preventing VSMC hyperpolarization. Oxidative escalation follows as ET-1-activated NADPH oxidase generates $O_2^-$ that fuels mitochondrial ROS production, converting residual NO to vasoconstrictive $ONOO^-$ while inducing eNOS uncoupling for further NO loss. Secondary injury cascades then manifest: neurovascular decoupling from nNOS degeneration lowers the threshold for cortical spreading depolarizations; ET-1-induced vasoconstriction converts these into "spreading ischemia"; and thromboinflammation erupts from lost NO's antiplatelet effects combined with ET-1's P-selectin induction. The resulting hypoxia perpetuates this cycle, rendering the brain increasingly vulnerable to energy supply–demand mismatch.

This self-reinforcing pathophysiology extends beyond macrovascular spasms to encompass capillary stalls, neutrophil plugging, and expanding infarct cores, all rooted in the ET-1/NO axis disruption where each molecule's dysfunction amplifies the other's downstream damage.

## 5. Pharmacological Interventions: Targeting the NO/ET-1 Axis

Pharmacological strategies targeting the NO/ET-1 axis restore vasodilatory–vasoconstrictive balance, inhibit oxidative stress/inflammation, and reverse vascular remodeling, offering core therapeutic approaches for cardiovascular/cerebrovascular and renal diseases. As shown in Table 1, the current drug development focuses on the following strategies.

**Table 1.** Pharmacological interventions targeting the NO/ET-1 axis.

| Strategy | Agents | Mechanism | Ref. |
| --- | --- | --- | --- |
| **NO Donors** | Sodium nitrite, L-Arginine | Hypoxia-triggered NO release; sGC activation | Østergaard L et al., 2013 [14]; Lilla N et al., 2016 [57] |
| **ET-1 Antagonists** | Clazosentan, Bosentan | Selective ETA blockade → ROS/eNOS uncoupling reversal | Macdonald RL et al., 2008 [91]; Galiè N et al., 2008 [92] |
| **NO Bioavailability Enhancers** | NAC, Glutathione | Scavenge ROS → reduce NO degradation, potentially reversing eNOS uncoupling | Kim M et al., 2023 [93] |
| **Multi-Target Agents** | HET0016, TS-011 | Multi-component synergy → restore NO-cGMP signaling, NO synthesis↑ | Tsai IJ et al., 2011 [94]; Miyata N et al., 2005 [95] |
| **Adjuvant Therapies** | Statins, Erythropoietin | Akt-eNOS phosphorylation → NO synthesis ↑ | Vergouwen MD et al., 2008 [96]; Vergouwen MD et al., 2010 [97] |
| **Emerging Approaches** | NOS gene therapy, Nanocarriers | Targeted eNOS delivery/activation | Khurana VG et al., 2002 [98]; Zhao YD et al., 2005 [99]; Taneja G et al., 2019 [100] |

For the restoration of the vasodilatory signaling, NO donors and analogs were widely considered. Classic nitrates like sodium nitroprusside could directly release NO, activating the sGC-cGMP pathway for rapid vascular smooth muscle relaxation. Clinical studies confirm that intrathecal administration significantly alleviates CVS post-SAH. Recently, successful therapeutic strategies against CVS have been applied using NO donors (L-Arginine, nitrite) and NO synthase gene therapy [13,14,80,101–103]. But, also, early NO donor treatment has been observed to increase CBF, e.g., due to preventing CTH increases, while, due to a hypotensive side effect, blood pressure and CPP are reduced, which are potentially beneficial effects due to hypertension often being a comorbidity in the presence of SAH [14,57–59]. Endogenous nitrite is converted to NO in the tissue without the need for oxygen as a substrate; thus, upon the infusion of nitrite, NO depletion and ROS production are reduced [14]. In addition, nitrite reduces mitochondrial proton leakage, increasing ATP yields from oxygen and thereby tissue tolerance to oxygen reduction [14], and may attenuate thrombogenicity by inhibiting thrombocyte aggregation [14]. Scientists have also tried to develop next-generation NO donors such as benzodifuroxan derivatives which can release NO via non-enzymatic pathways, generating reactive nitrogen species (e.g., $NO^-$) that efficiently activate sGC even in hypoxic tissues, thus overcoming drug resistance [104,105]. Inorganic nitrites like sodium nitrite could also be converted to NO by tissue reductases without oxygen dependence, enhancing hypoxic tolerance. SAH models show 15–30% increased CBF and reduced mitochondrial proton leakage to boost ATP synthesis. Still, systemic hypotension (>25% incidence) and tachyphylaxis (50% efficacy loss after chronic use) remain key challenges in the clinical trials.

ET-1 receptor antagonists were also considered as an effective pharmacological intervention approach, as they can efficiently block vasoconstrictive signaling [106]. $ET_A$ antagonists such as Ambrisentan can specifically block $ET_A$ receptors, inhibiting ET-1-mediated vasoconstriction/smooth muscle proliferation. It has already been used for pulmonary arterial hypertension (PAH) but carries peripheral edema risk (28.6% incidence) [92]. Clazosentan could reduce CVS incidence by 43% in SAH Phase II trials but failed to significantly improve delayed cerebral ischemia outcomes [91]. Dual $ET_A/ET_B$ antagonists were also

considered helpful in this approach. For example, Macitentan/Sparsentan can block $ET_A$ (inhibit constriction), while partially activating $ET_B$ (promoting NO release) [107]. Sparsentan has been shown to be able to reduce the urine albumin–creatinine ratio (UACR) by 49.5% in diabetic nephropathy trials vs. 33.1% with angiotensin receptor blockers (ARBs) [108]. Advanced delivery systems were also developed to produce a sustainable supply of the ET-1 receptor antagonists. For example, the PER-001 intravitreal implant, which can make a sustained release of $ET_A$ antagonist within a 6-month duration, has been applied in a diabetic retinopathy Phase 2a trial. Results from this trial indicate that the treatment significantly reduced macular ischemia and improved low-luminance visual acuity [109].

Beyond directly supplementing NO or blocking ET-1 receptors, a complementary therapeutic strategy aims to enhance endogenous NO bioavailability by targeting the underlying oxidative stress. In this context, thiol-based antioxidants such as N-acetylcysteine (NAC) and glutathione present a promising approach [93]. These agents do not directly donate NO but instead act as potent scavengers of ROS, thereby shielding NO from premature degradation and potentially reversing eNOS uncoupling. This restoration of the redox balance helps to preserve the integrity of the NO-sGC-cGMP signaling pathway, effectively counteracting ET-1-induced vasoconstriction and endothelial dysfunction from a different nodal point. This strategy is particularly relevant in the highly oxidative environment of SAH, where the efficacy of conventional agents like L-arginine can be compromised.

Pharmacological targeting of the NO/ET-1 axis is evolving from single-pathway modulation to multi-pathway coordination. As shown in Table 2, over the recent decades, scientists have also focused on the development of multi-target agents modulating NO/ET-1 balance, each with a unique mechanism to restore the NO-cGMP signaling. A prime example is the 20-HETE synthesis inhibitor TS-011 [44]. By blocking the production of 20-HETE, it not only prevents hemoglobin-induced vasospasm but, more importantly, demonstrates the capability to reverse established vasospasm when administered several days post-SAH, normalizing the vascular diameter [44]. Furthermore, by antagonizing specific G protein-coupled receptors for 20-HETE such as GPR75, significant drivers of hypertension and vascular dysfunction, such as the CYP/20-HETE/GPR75 axis, could be effectively suppressed [110]. Thus, this approach presents a promising strategy to block the deleterious effects of 20-HETE, including vascular inflammation, endothelial dysfunction, and the sensitization to constrictor stimuli, without affecting its synthesis, offering a novel means to rebalance the NO/ET-1 axis and mitigate cerebrovascular diseases.

**Table 2.** Representative multi-target agents modulating NO/ET-1 homeostasis.

| Drug Class | Representatives | Mechanism | Ref. |
| --- | --- | --- | --- |
| **ACEi/ARBs** | Enalapril | Inhibit Ang II → ↓ET-1 synthesis + ↑NO release | Elmarakby AA et al., 2003 [111] |
| **sGC Stimulators** | Riociguat | Directly activate sGC (NO-independent) → ↑cGMP | Meis T et al., 2014 [112] |
| **20-HETE Inhibitors** | HET0016, TS-011 | Inhibit CYP4A → restore NO-cGMP signaling, reverse pericyte constriction | Kehl F et al., 2002 [113]; Cambj-Sapunar L et al., 2003 [114]; Tsai IJ et al., 2011 [94]; Benter IF et al., 2005 [115]; Takeuchi K et al., 2005 [44] |
| **Herbal Formulations** | Ligusticum chuanxiong | Multi-component synergy: ↓ET-1/IL-5 + ↑NO | Wang C et al., 2006 [116]; Seo Y et al., 2020 [117] |

Of note, herbal active components such as Tetramethylpyrazine (TMP) from Ligusticum chuanxiong have demonstrated the ability to suppress cerebral vasospasm by upregulating eNOS activity and inhibiting ET-1 overproduction, synergizing with nimodipine to improve cerebral blood flow in SAH models [116,117]. Besides these approaches, in-

novative combination strategies and delivery systems were also investigated to further improve the therapeutic potential. Sequential combination therapy, such as the PAH protocol, combines ERA (e.g., Macitentan) and phosphodiesterase (PDE)-5i (e.g., Sildenafil) for the low-risk group, while using Prostacyclin for the high-risk group, significantly improving the 5-year survival rate to over 75% [118]. Researchers also focused on the SAH window; early nitrite (anti-hypoxia) and delayed ETA antagonist (anti-remodeling) were selectively suggested to avoid hypotension [119–121]. Nanodelivery systems such as the liposome-encapsulated eNOS gene or eNOS-activating nanoparticles targeting cerebrovascular endothelia have been considered to increase local NO production [100]. Personalized therapies which stratify patients by their individual ET-1/NO ratio or ADMA (endogenous NOS inhibitor) levels might be recommended for precision dosing [122]. Emerging targets in this pharmacological field also include sGC allosteric activators such as BAY 58-2667, which can activate oxidized sGC and is effective in high-oxidative SAH conditions [123], as well as ETB-biased agonists which can selectively stimulate the ETB-NO pathway without ETA-mediated side effects [124].

Yet, for further drug development, certain existing pharmacokinetic barriers still limit the clinical translation outcome. For example, a low BBB penetration limits many ET-1 antagonists from reaching the central nervous system (CNS), limiting CVS/DCI efficacy. The short NO donor half-life also demands more sustained-release formulations. Future breakthroughs will rely on innovative drug design (e.g., long-acting NO donors), precision delivery (e.g., brain/ocular implants), and phenotype-guided combinations to achieve comprehensive benefits—from vasospasm relief to end-organ protection.

## 6. Discussion

The emerging evidence suggests that the bidirectional regulation of NO and ET-1 represents a central pathogenic axis in the development of CVS and DCI following SAH. As reviewed, the imbalance between vasodilatory NO and vasoconstrictive ET-1 drives microcirculatory dysfunction, pericyte constriction, oxidative stress, and neurovascular uncoupling. Pharmacological strategies aimed at restoring this balance such as NO donors, ET-1 receptor antagonists, and multi-target agents like 20-HETE inhibitors have shown promise in preclinical and early clinical studies. However, the translational success of these approaches has been limited by challenges such as systemic hypotension, poor blood–brain barrier penetration, and the complexity of NO signaling dynamics in the ischemic brain.

A critical layer of complexity arises from the dual pathways of NO generation. Beyond the canonical NOS-dependent pathway [125], the NOS-independent nitrate–nitrite–NO pathway serves as a vital backup system, particularly under hypoxic conditions prevalent in SAH [126]. While the canonical pathway is compromised by ET-1-mediated inhibition, ROS-induced eNOS uncoupling, and oxygen scarcity, the reduction of nitrite to NO by deoxyhemoglobin and other 5-coordinated ferrous hemes (e.g., in cytochromes) is enhanced in hypoxia [127]. Importantly, the substrate nitrite is readily available from plasma and brain tissue, and can be replenished from dietary nitrate via microbial reduction in the oral cavity and gut, establishing a clinically accessible "enterosalivary nitrate-nitrite-NO" axis [128]. Under the specific SAH pathophysiological conditions, leveraging the nitrite–NO pathway represents a strategic bypass to restore NO bioavailability despite high ET-1 tones. This explains the efficacy of sodium nitrite in experimental SAH, improving CBF without relying on compromised NOS activity, probably also attenuating mitochondrial dysfunction [14,129–131]. This NOS-independent pathway may serve as a critical backup mechanism to maintain NO bioavailability when the canonical pathway is impaired, offering a promising therapeutic avenue for conditions like SAH where hypoxia and NOS dysfunction coexist.

Therefore, future therapeutic strategies should not only target the NO-ET-1 axis via conventional NOS modulation or ET-1 antagonism but also exploit the nitrite–NO pathway to bypass NOS limitations for the optimal outcomes. Interventions such as nitrite infusion, hypoxia-activated NO donors, or agents that enhance nitrite reduction could provide sustained NO delivery without exacerbating oxidative stress or depending on oxygen availability.

## 7. Conclusions

The NO-ET-1 axis plays a pivotal role in the pathophysiology of cerebral vasospasm and delayed cerebral ischemia after SAH. While the current therapeutic approaches focus on rebalancing this axis through NO donors, ET-1 antagonists, and multi-target agents, their clinical efficacy remains partial. A deeper understanding of NO biology offers new insights and opportunities for intervention. By integrating strategies that enhance both canonical and alternative NO sources, and by timing interventions to align with the evolving pathophysiology of SAH, we may better address the multifactorial nature of CVS and DCI. Future research should prioritize the development of targeted, hypoxia-sensitive NO delivery systems and personalized treatment regimens based on individual NO/ET-1 profiles and redox status, ultimately improving outcomes for patients suffering from this devastating condition.

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| 20-HETE | 20-Hydroxyeicosatetraenoic Acid |
| $\alpha$-SMA | alpha Smooth Muscle Actin |
| ARBs | Angiotensin Receptor Blockers |
| ACEi | Angiotensin-Converting Enzyme Inhibitors |
| AngII | Angiotensin II |
| ADMA | Asymmetric Dimethyl Arginine |
| BBB | Blood–Brain Barrier |
| CBF | Cerebral Blood Flow |
| cGMP | Cyclic Guanosine Monophosphate |
| CNS | Central Nervous System |
| $CO_2$ | Carbon Dioxide |
| CSD | Cortical Spreading Depolarizations |
| CSI | Cortical Spreading Ischemia |
| CVS | Cerebral Vasospasm |
| CTH | Transit Time Heterogeneity |
| CYPA4 | Cytochrome P450 |

| | |
|---|---|
| DCC | Deleted in Colorectal Cancer |
| DCI | Delayed Cerebral Ischemia |
| DIND | Delayed Ischemic Neurological Deficit |
| eNOS | Endothelial NOS |
| ERK | Extracellular Signal Regulated Kinase |
| ET-1 | Endothelin-1 |
| HO-1 | Heme Oxygenase-1 |
| iNOS | Inducible NOS |
| I/R | Ischemia/Reperfusion |
| KCa channels | Calcium-Activated $K^+$ Channels |
| L-NAME | L-$N^G$-Nitro Arginine Methyl Ester |
| MAPK | Mitogen Activated Protein Kinase |
| metHb | Methaemoglobin |
| MMP9 | Matrix Metalloproteinase 9 |
| MnSOD | Mangan Superoxide Dismutase |
| MTT | Mean Transit Time |
| NADPH | Nicotinamide Dinucleotide Phosphate |
| NF-κB | Nuclear Factor Kappa B |
| NMDA | N-Methyl-D-Aspartate |
| NO | Nitric Oxide |
| NOS | Nitric Oxide Synthase |
| nNOS | Neuronal NOS |
| $O_2^-$ | Superoxide |
| $ONOO^-$ | Peroxynitrite |
| oxyHb | Oxygenated Hemoglobin |
| PAH | Pulmonary Arterial Hypertension |
| PARP | Poly(Adenosine Diphosphate-Ribose)-Polymerase |
| PDE | Phosphodiesterase |
| PI3K/Akt | Phosphatidylinositol-3-Kinase/Protein Kinase B |
| PKG | Protein Kinase G |
| ROCK | Rho-Associated Protein Kinase |
| ROS | Reactive Oxygen Species |
| SAH | Subarachnoid Hemorrhage |
| sGC | Soluble Guanylate Cyclase |
| TMP | Tetramethylpyrazine |
| tPA | Tissue Plasminogen Activator |
| VEGF | Vascular Endothelial Growth Factor |
| VSMC | Vascular Smooth Muscle Cell |

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